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THE JOURNAL OF PARASITOLOGY

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The Journal of Parasitology

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STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXXVI. PARASITES OF THE WOLVERINE, *GULO GULO* L., WITH OBSERVATIONS ON THE BIOLOGY OF *TAENIA TWITCHELLI* SCHWARTZ, 1924

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Natural biotic relationships already had been severely disrupted in the United States by the time significant interest had developed in faunistic helminthology. Some mammalian species, particularly the larger carnivores, had been extirpated or were represented only by scattered individuals in the few remaining wilderness areas. Thus, it is not remarkable that the helminths of such species as the wolverine, *Gulo gulo* Linnaeus, have been little studied. Fortunately, however, much of arctic and subarctic North America has endured in its primitive state, and here it is still possible to undertake basic biological studies under undisturbed conditions.

A comparatively large series of wolverines has been examined in this laboratory since 1949, and the helminths commonly occurring in this mammal in Alaska have been identified. Supplemental observations have been made on the anatomy of *Taenia twitchelli* Schwartz, 1924, and new information has been acquired on the life cycle of this cestode. The results of this work are reported herein.

MATERIALS AND METHODS

Eighty wolverine carcasses suitable for detailed examination were obtained from hunters and trappers, thus obviating the need for killing any animals solely for this investigation. The map (fig. 1) indicates locations from which the animals originated. Excepting one immature animal captured in July, all were taken during the colder months (October through May).

After first being isolated in tap water, the cestodes were fixed in hot 10 percent formalin, dehydrated in ethanol, cleared in terpineol, and prepared as permanent mounts. The nematodes were fixed in hot 70 percent ethanol, cleared in lactophenol, and studied unmounted. To establish experimental infections, eggs were taken from gravid segments of living cestodes and, by means of a pipette, introduced into the stomachs of experimental animals in a state of ether-induced anesthesia. Tissues collected from such animals were fixed in 10 percent formalin, embedded by the paraffin method, and sections were stained in hematoxylin-eosin.

RESULTS

Helminths were found in 69 (86 percent) of the 80 wolverines. Each species is discussed separately below.

TREMATODA

Genus *Alaria* Schrank, 1788

Alaria sp.: Fecal smears from a captive wolverine were examined daily for 7 weeks in connection with an experimental *Taenia* infection. Two trematode eggs were observed in one smear and, 39 days later, when the animal was autopsied, a

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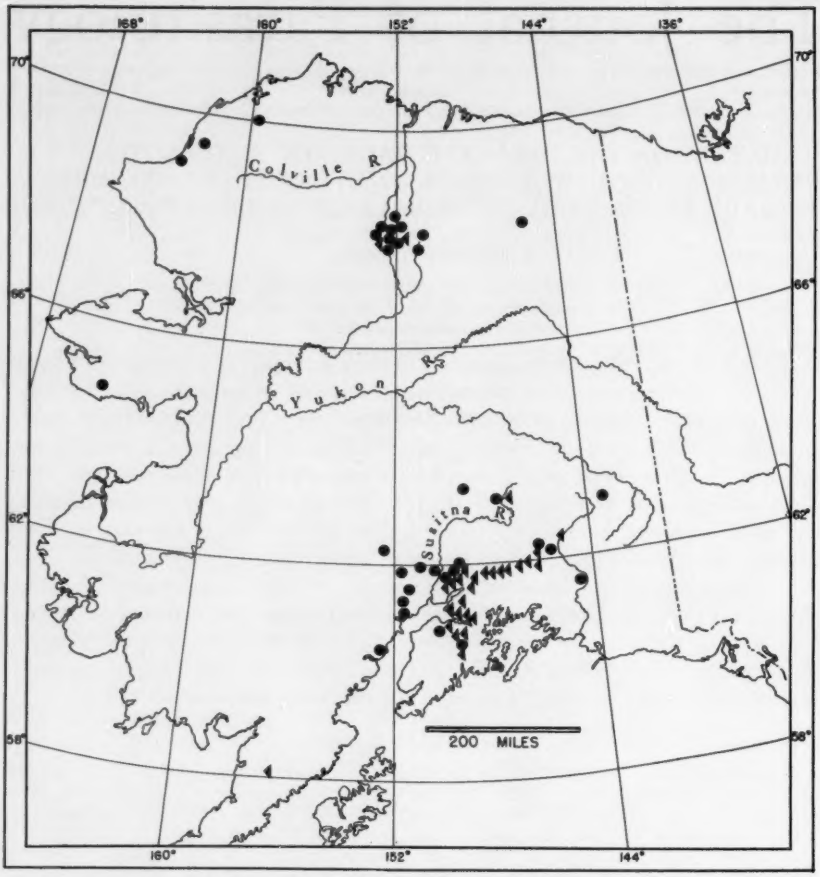


FIGURE 1. Map of Alaska, showing locations from which wolverines (circles) and porcupines (triangles) were obtained.

single trematode of the genus *Alaria* was found in the small intestine. This wolverine, not included in the above total, had been in captivity for about 2 years, during which time its diet precluded accidental infection by helminths.

The trematode differs morphologically from the known species of *Alaria,* none of which has been reported from wolverines, but a detailed study is deferred until additional material can be obtained.

CESTODA

Genus *Mesocestoides* Vaillant, 1863

Mesocestoides kirbyi Chandler, 1944: This species was collected from 2 (2.5 percent) of the 80 wolverines, and of these, one captured near Willow, south-central Alaska, had 2 cestodes similar in size to specimens from coyotes, *Canis latrans* Say, in Alaska. Ten smaller strobilae were found in a second wolverine, killed near Moose Pass, on the Kenai Peninsula. *M. kirbyi* is the only species of the genus

known to occur in mammals in Alaska; material from canine hosts has been studied by Voge (1955). Species of *Mesocestoides* have not previously been recorded from wolverines.

Genus *Taenia* Linnaeus, 1758

Taenia twitchelli Schwartz, 1924: *T. twitchelli* was found in 60 wolverines (75 percent). In 27 infected animals these cestodes ranged in number from 1 to 61, with an average of 17. The life cycle and host relationships of *T. twitchelli* are discussed below.

NEMATODA

Genus *Trichinella* Railliet, 1895

Trichinella spiralis (Owen, 1835): Larval nematodes identified as *T. spiralis* were found in 19 (49 percent) of 39 wolverines appropriately examined. The occurrence of *T. spiralis* in this and other species of mammals in Alaska has been discussed elsewhere (Rausch et al, 1956).

Genus *Molineus* Cameron, 1923

Molineus patens (Dujardin, 1845): A single male specimen of this trichostrongyle was obtained from the small intestine of a wolverine killed near Willow and, although *M. patens* has been reported from other mustelids, this appears to be the first North American record from this host. In Eurasia, Machul'skii (1953) found *M. patens* in one of 4 wolverines from Buriat-Mongolia.

Genus *Ascaris* Linnaeus, 1758

Ascaris devosi Sprent, 1952: One of the more common helminths observed, *A. devosi* was recorded from 17 (21 percent) of the wolverines. It was found in 12 (30 percent) of 39 animals from the Brooks Range, Arctic Alaska. The other 5 were collected farther south. Individual animals had from one to 13 ascarids, with an average of about 6.

Although *A. devosi* is the only species of *Ascaris* known to occur in the wolverine in North America, the identity of the ascarids from this host in Eurasia appears to be questionable. Nematodes identified as *A. columnaris* Leidy, 1856, have been reported by Oshmarin (1946; cited in Machul'skii, 1953) from wolverines in Buriat-Mongolia. Spasskii et al (1952) recorded *A. columnaris* from this host in the region of Lake Baikal, and Machul'skii (1953) found it in 1 of 4 animals examined in Buriat-Mongolia. As pointed out by Sprent (1952), the morphological differences between *A. devosi* and *A. columnaris* might have been overlooked, and *A. devosi* may actually be the species occurring in Eurasian wolverines. Sprent (1952) also reviewed the status of other species of ascarids that have been reported from various mustelids in Eurasia.

Sprent (1953) concluded that an intermediate host (rodent) may be obligatory in the life cycle of *A. devosi*. In Alaska, both microtine rodents and smaller species of sciurids might serve as intermediate hosts of this nematode.

Genus *Physaloptera* Rudolphi, 1819

Physaloptera torquata Leidy, 1856: *P. torquata* was recorded from only 4 wolverines (5 percent), all from the Brooks Range region. One was taken near Anak-

tuvuk Pass, and 3 were killed in the taiga biome near Arctic Village. One of the latter contained 17 specimens, the maximum seen in a single animal.

Although known to occur in other North American species of mustelids farther to the south, this is the first record of *P. torquata* from the wolverine. *P. sibirica* Petrov and Gorbunov, 1931, has been reported from wolverines in Eurasia (Oshmarin, 1946; Spasskii et al, 1952).

Observations on Taenia twitchelli

T. twitchelli was described by Schwartz (1924) from larvae found in the lungs and pleural cavities of a porcupine, *Erethizon dorsatum myops* Merriam, from Ophir, Alaska. A description of the adult stage was later provided by McIntosh (1938), who obtained specimens from a wolverine killed at Rapids, east-central Alaska. There are no further published records of the occurrence of this cestode.

I. The Adult Cestode

McIntosh (1938) remarked that his 3 cestodes were contracted, did not stain well, and also lacked gravid segments. It therefore seems desirable to provide a more complete description of this tapeworm.

There is an unusual tendency for strobilae of *T. twitchelli* to contract, making it difficult to obtain specimens suitable for detailed study. Those dying in situ in the host are usually so contracted as to convey the impression that *T. twitchelli* differs morphologically from its congeners much more than is actually the case. The following description of the adult stage is based on specimens removed alive, allowed to relax, and fixed. Complete relaxation of the anterior portion of the strobila often cannot be accomplished, even under the most favorable conditions.

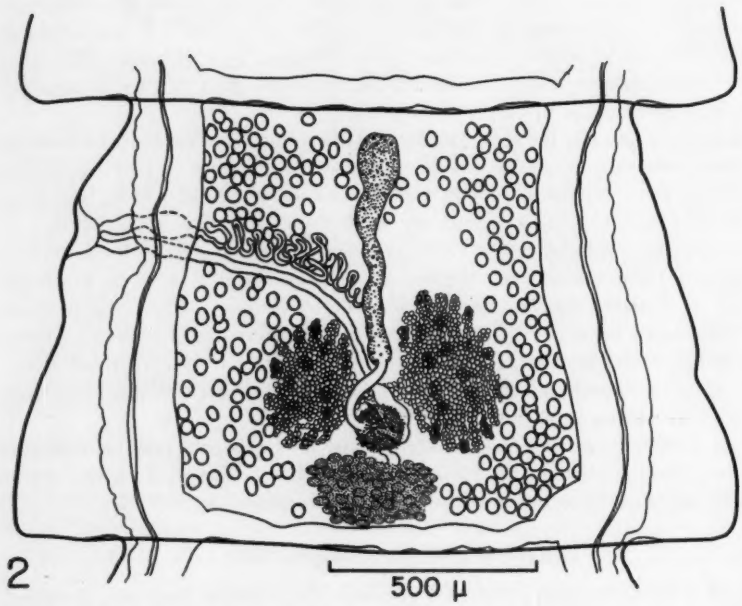
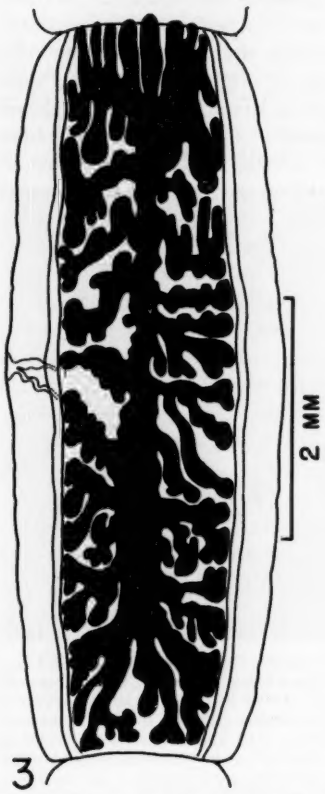


FIGURE 2. Mature segment of *Taenia twitchelli*, ventral view.

Taenia twitchelli Schwartz, 1924 (figs. 2–4)

Diagnosis: Strobila up to 210 mm long, with a maximum of 120 segments. Anterior segments wider than long; in partially contracted strobilae this anterior region comprises as much as ¼ of strobilar length, and may contain more than half the total number of segments. Length/width ratio of segments increases toward posterior end of strobila. Mature segments trapezoidal and slightly longer than wide; gravid segments 2 to 3 times as long as wide. Maximum width attained in pre-gravid segments. Strobilar margins markedly serrate, particularly in region of mature segments. Scolex relatively small, measuring up to 1.2 mm in diameter; only slightly set off from unsegmented neck. Suckers small, about 265 microns in diameter. Rostellum armed with 32 to 36 hooks arranged in 2 rows; large and small hooks measure 200 to 212 microns and 156 to 168 microns in length, respectively. Genital pores irregularly alternate, situated in anterior half of segment. Genital ducts dorsal to longitudinal excretory canals. Elongate cirrus sac extends across ventral longitudinal excretory canal, and measures about 220 by 80 microns in mature segments. Vas deferens much coiled at proximal end of cirrus sac. Testes, about 200 per segment, average about 50 microns in diameter in mature segments; they are arranged in 2 lateral fields which may be confluent across anterior portion of segment, extending posteriad to level of posterior margin of vitelline gland, and some may slightly overlap ventral longitudinal excretory canals. Vagina opens into genital atrium just posterior to cirrus sac, runs transversely mediad, curving slightly posteriad, and enlarges to form small seminal receptacle between ovarian lobes and usually dorsal to Mehlis' gland. Ovary situated immediately posterior to center of segment; it consists of 2 reniform lobes, of which the larger is aporal. Vitelline gland lies posterior to ovary, in notch formed by margins of ovarian lobes; it approximates larger ovarian lobe in size and shape. Mehlis' gland about 100 microns in



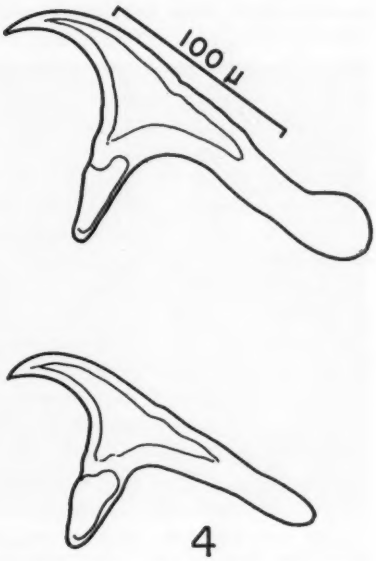


FIGURE 3. Gravid segment of *T. twitchelli.*
FIGURE 4. Rostellar hooks of *T. twitchelli.*

diameter in mature segments. In terminal segments, gravid uterus usually has 10 to 12 lateral branches on each side; these subdivide, usually filling segment within limits of longitudinal excretory canals; branches at anterior end of gravid uterus usually extend slightly into preceding segment. Eggs measure from 30 to 36 microns in diameter (av. 34 microns).

Host: Wolverine, *Gulo gulo* Linnaeus.
Habitat: Duodenum.
Distribution: Boreal North America.

The characteristics of the rostellar hooks serve to distinguish the adult *T. twitchelli* from both *T. mustelae* Gmelin, 1790, and *T. martis* (Zeder, 1803), the only other species of *Taenia* known from mustelids in boreal regions (cf. Freeman, 1956).

T. twitchelli has been reported only from Alaska. I am indebted to Dr. Ian McTaggert Cowan, University of British Columbia, Vancouver, for permission to publish his record of this species from a wolverine killed on May 31, 1945, at Topaz Lake, Jasper National Park. It is probably a parasite of the wolverine throughout its range in North America.

II. The Larval Cestode

According to Schwartz (1924, p. 1), the description of *T. twitchelli* was based upon "... two detached cysticerci and a number of cysticerci attached to the lung tissue, some being attached directly to the lungs by means of a peduncle that penetrates into the lung tissue and others being attached indirectly, the peduncles of the individual cysticerci converging to a more or less common origin from which a stalk penetrates into the lung substance." Although these larvae had fully developed rostellar hooks, they were much smaller and differed in form from those I have obtained from both naturally and experimentally infected porcupines. Some of these differences may be due to the fact that Schwartz' specimens were preserved in brine.

Since 1953, 89 mammals representing 12 species, listed in table I, have been exposed to experimental infection. In addition, several animals received eggs later determined to have been non-infective. The smaller species were laboratory reared, but the porcupines, muskrats, and squirrels were trapped near Anchorage.

Development in Experimental Animals: Although the porcupine appears to be the natural intermediate host of *T. twitchelli,* larval development was ostensibly normal in experimentally infected animals of other species. Since rodents of species available in numbers for experimental use were found to resist infection, it was not possible to follow larval development to the infective stage in mammals of one kind. Animals killed by the infection were autopsied at once; otherwise experimental animals were killed at intervals for examination. The findings in selected animals are presented in chronological sequence. The various periods of lapsed time mentioned were computed from the day of exposure. No observations were made prior to the 9th day in susceptible species.

9th day: Two voles, *Microtus pennsylvanicus,* died on the 9th day after each had received more than 1000 eggs. Macroscopically, the lungs showed many white foci, and no normal-appearing tissue was visible. In slices of lung stained and cleared in toto, the larvae appeared as translucent, thin-walled bodies varying in shape from somewhat elongate to nearly sperical, and up to 500 microns in greatest length. In tissue sections, they appeared oval to round in cross-section; the well-defined cuticle was lined by a thin layer of cells enclosing an acidophilic reticulum containing scattered cells of large size.

13th day: A porcupine that had received an entire gravid segment died on the morning of the 13th day. The pulmonary surfaces showed many white, subpleural foci, some of which appeared as discrete streaks up to 7 mm long (fig. 5). In some foci, larvae were visible immedi-

ately below the pleura. Stained and cleared cross sections cut parallel with the pulmonary surface revealed that the focal lesions had resulted from larval migration toward the pleura; or, in the case of the streaks, along the pulmonary surfaces just beneath the pleura. At this time, the larvae were ovoid to pyriform in shape, and as much as 1.3 mm in length. They occurred singly at the more superficial ends of the streaks; when pyriform in shape, the larger (anterior) end was directed outward. The wall of the anterior end usually appeared somewhat thicker than in the remainder of the body. None had penetrated the pleura, although some had caused it to bulge slightly. Most of the larvae lay at the ends of leukocyte-filled tunnels they had made through the tissue, and were in contact with normal tissue through which they were making their way.

TABLE I. Results of feeding eggs of *T. twitchelli* to experimental animals.

Species	Number exposed	Number infected
Larval development ostensibly normal:		
Ground squirrel, *Citellus undulatus* Pallas	3	3
Red squirrel, *Tamiasciurus hudsonicus* (Erxleben)	1	1
Brown lemming, *Lemmus sibiricus* (Kerr)	7	2
Field vole, *Microtus pennsylvanicus* (Ord)	2	2
Muskrat, *Ondatra zibethicus* (Linnaeus)	5	5
Porcupine, *Erethizon dorsatum* (Linnaeus)	7	7
Larval development inhibited or abnormal:		
Hamster, *Mesocricetus auratus* Waterhouse	4	—
Red-backed vole, *Clethrionomys rutilus* Pallas	45	—
Tundra vole, *Microtus oeconomus* Pallas	6	—
White rat, *Rattus norvegicus* (Berkenhout)	2	—
White mouse, *Mus musculus* Linnaeus	6	2
Rhesus monkey, *Macaca mulatta* Zimmermann	1	—

17th day: A red squirrel that had received a gravid segment behaved normally on the 15th day, showed signs of severe respiratory distress on the 16th day, and died in the afternoon of the 17th day. The pleural cavities contained a quantity of sero-sanguineous fluid in which 419 free larval vesicles were found. These, after becoming somewhat turgid in water, averaged about 10 mm in length, with a maximum length of 15 mm. Nearly all had an irregular shape, due to the formation of buds (fig. 6). The walls of the vesicles showed small, disc-shaped aggre-

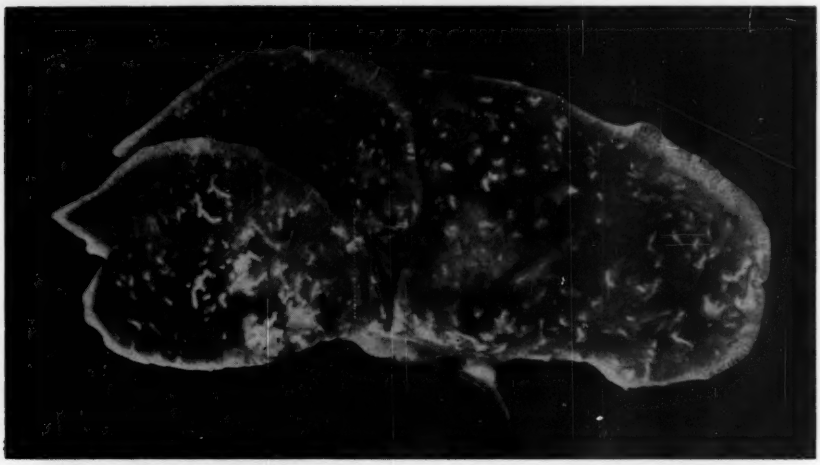


FIGURE 5. Lung of porcupine on 13th day after exposure, dorsal view; location of migrating larvae of *T. twitchelli* indicated by white foci.

gations of cells that were visible as small opacities when unstained. These aggregations, the primordia of the scolices, occurred at the apices of the buds, some of the largest of which already showed beginning invagination at the tip. A few vesicles protruded partially through the pleura, and both lungs showed areas of massive hemorrhage (fig. 7). A few larvae were found in the liver, but none had emerged. Sections showed a few also still deep within the pulmonary tissue; in these, the cuticle had a thickness of about 6 microns, and was lined by a layer of cells about 12 microns thick. The lumina of these larvae were filled by a delicate, acidophilic reticulum. The

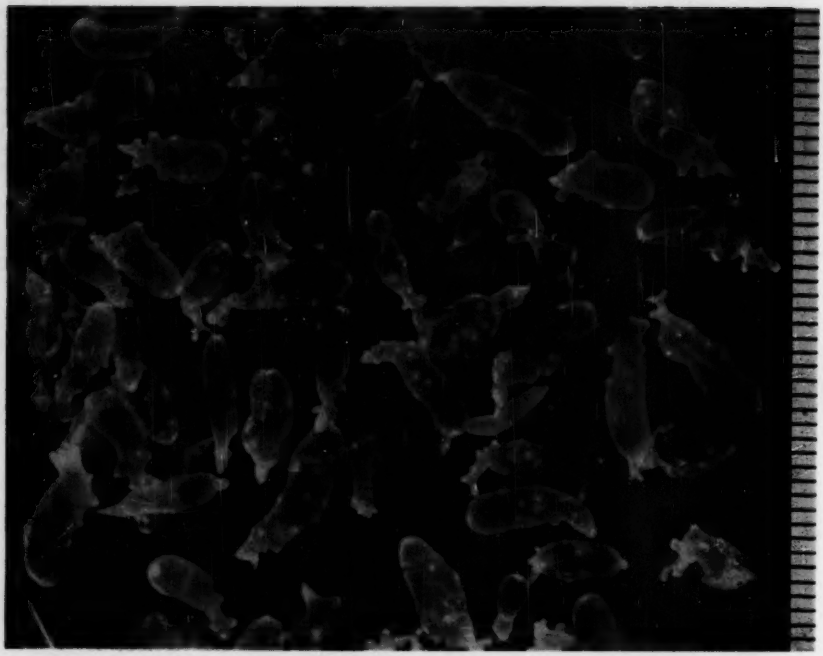


FIGURE 6. Larvae of *T. twitchelli* from the pleural cavities of a red squirrel on the 17th day; primordia of scolices and early buds visible. (Each space of scale has value of 1 mm.)

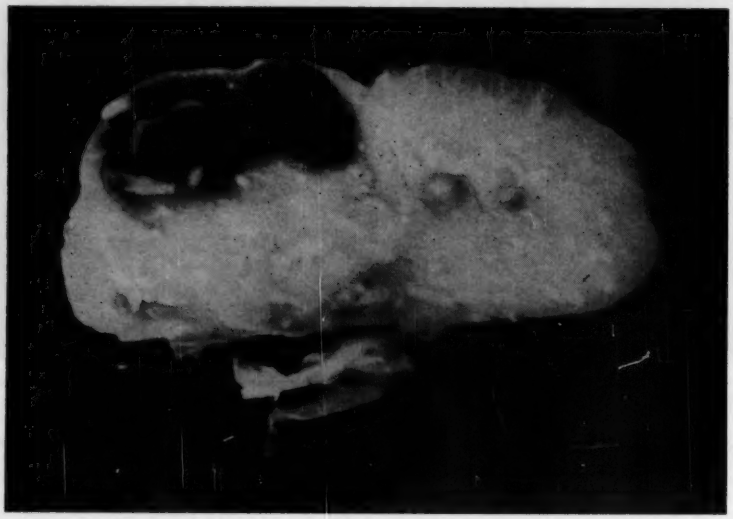


FIGURE 7. Lung of red squirrel on 17th day, dorsal view, showing hemorrhagic area and superficial lesions caused by emergence of larvae of *T. twitchelli*.

larvae in the liver were identical in form to those in the lungs.

20th day: A large adult porcupine died on the 20th day after receiving several thousand eggs. Macroscopically, the lungs appeared to be involved to about the same degree as in the porcupine that died on the 13th day, but the pleura was considerably thickened. Numerous larvae protruded through the pleura, and 200 were found free in the pleural cavities; these averaged smaller than those from the red squirrel mentioned above, but the buds were somewhat more advanced in development. Larvae were numerous in the liver of this animal, and often were found in small, blood-filled cavities. None had penetrated the hepatic peritoneum. Tissue sections showed larvae to be still numerous in the lungs.

24th day: In a porcupine that died on the 24th day, the lungs were involved to about the same extent as those from the porcupines on the 13th and 20th days, but the effects of the chronic course of the infection were more evident. The pleura was so much thickened and so opaque that the underlying lesions were hardly visible. The right lung was the more severely affected, and the right pleural cavity contained a large, stratified blood-clot as well as 30 ml of sero-sanguineous fluid. There were blood-clots in the left pleural cavity also, but little fluid. Exclusive of those trapped in the clots, 91 free larvae were found in the pleural cavities. They resembled those found on the 20th day, but with buds more advanced in development. Many larvae remained within the lungs.

60th day: A muskrat that had received small numbers of eggs (fed in the feces of an infected wolverine) over a period of 4 consecutive days died on the 60th day after the first feeding. The lungs showed many white foci, and the pleura was much thickened. A few larvae were partially emerged from the ventral pulmonary surfaces, and 678 free vesicles completely filled the pleural cavities. The larvae ranged up to 20 mm in length and, in addition to buds in various stages of development, some had produced secondary vesicles by means of exogenous proliferation (fig. 8). Many of the latter had already become detached from the primary vesicles, and

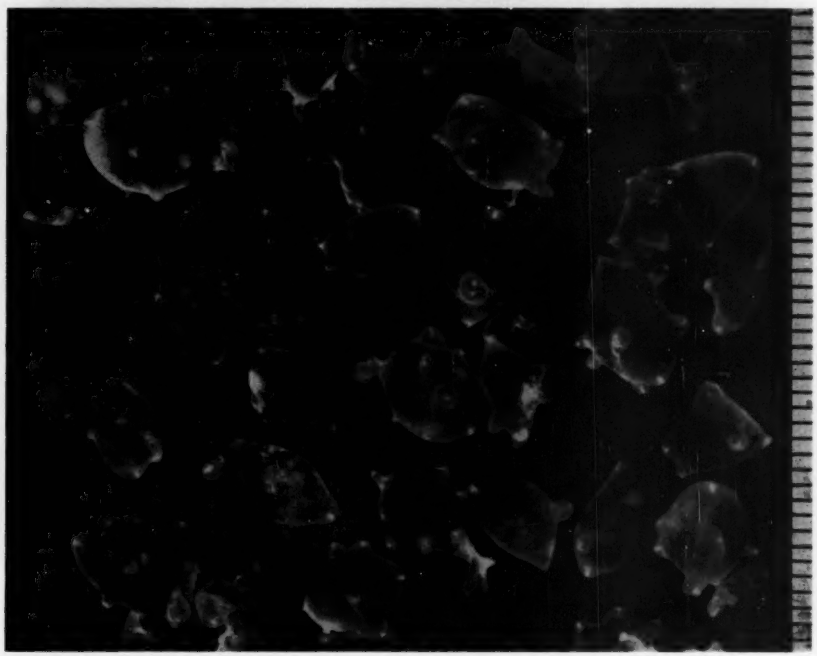


FIGURE 8. Larvae of *T. twitchelli* from the pleural cavities of a muskrat on the 60th day after exposure; buds well advanced, some larvae having produced secondary vesicles. (Each space of scale has value of 1 mm.)

were undergoing independent development. Most did not yet show the primordia of scolices. The most advanced buds on the larger primary vesicles had deep, apical invaginations (as much as 600 microns in depth), but the formation of rostellar hooks was not yet apparent. Sections disclosed a few apparently immobilized vesicles, surrounded by a narrow zone of connective tissue, still deep within the pulmonary tissue. Although irregular in shape, these had produced no buds. Ten larvae, resembling the largest from the pleural cavities, were found in the abdominal cavity.

63rd day: A single vesicle was found in the pleural cavity of a brown lemming killed on the 63rd day. Although measuring only 6 mm in diameter, it had 7 short peduncles, each with a well-developed scolex and fully developed hooks.

67th day: A muskrat received eggs on 2 consecutive days, and died on the 67th day after the first feeding. This death was not definitely attributable to the infection, since few larvae were present. Thirteen large vesicles were found in the pleural cavities; 8 of these were free, and 5 were attached to the pulmonary pleura in the vicinity of the hili. All possessed large scolices on long peduncles, and the rostellar hooks were fully developed. Nine similar larvae were found in the abdominal cavity.

91st day: Another muskrat given eggs on 2 successive days was examined on the 91st day after the first feeding. Macroscopically, the lungs appeared normal, and the pleural cavities were found to contain 2 vesicles with 2 and 6 scolices, respectively. These were fed to a captive wolverine, and not studied in detail.

94th day: A single larva with 3 mature scolices was found in the pleural cavity of a brown lemming examined on the 94th day.

96th day: A muskrat that had received eggs over a period of 4 successive days was killed on the 96th day. Macroscopically, the lungs of this animal appeared normal. Of the 10 larvae found in the pleural cavities, 1 was attached to the tissue near the hilus, on the left side, and 9 were free. These larvae each had several elongate peduncles, and the rostellar hooks were fully developed (fig. 9). Nine additional larvae were found in the abdominal cavity.

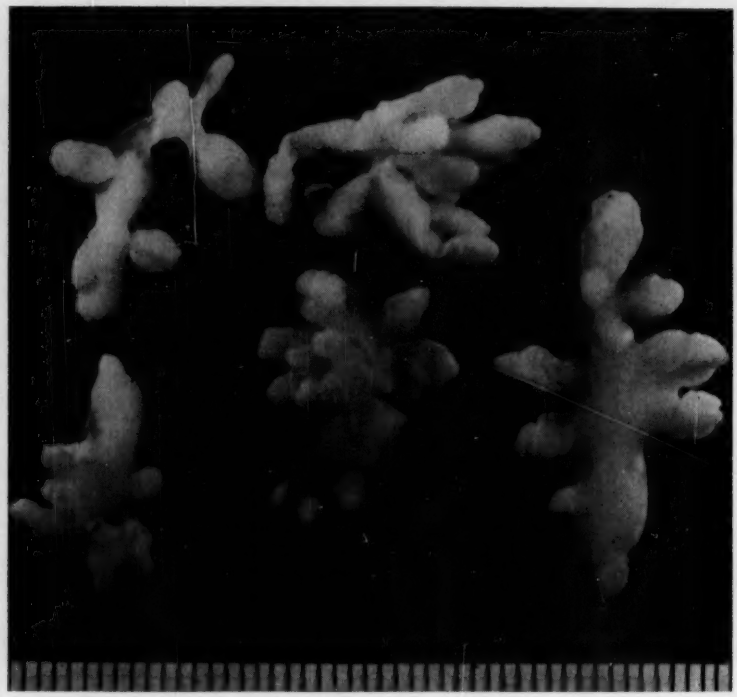


FIGURE 9. Infective larvae of *T. twitchelli* from pleural cavities of a muskrat on the 96th day after exposure. (Each space of scale has value of 1 mm.)

109th day: On the 109th day, a ground squirrel died of pulmonary disease caused, at least primarily, by the infection. It showed signs of severe respiratory distress on the 108th day, although its physical condition had deteriorated over a period of several weeks. Macroscopically, large portions of the lungs appeared atelectatic; numerous white foci were present, and the pleura was covered by a layer of fibrino-purulent exudate. Larval vesicles, 247 in number, completely filled the pleural cavities; these showed a wide range of developmental stages, with the largest measuring up to 27 mm in length. Some of the smaller vesicles showed only the early primordia of scolices, with no budding, and presumably had been produced by exogenous proliferation, while some were mature. A large larva was found within the pericardium, which was much thickened. Several with mature scolices were present in the abdominal cavity; one

mass was attached near the base of the uterus, others were in the omentum and mesenteries, and some were free. One was attached retroperitoneally on the ventral surface of the psoas muscles. There was a general peritonitis, with adhesions between the liver and diaphragm. In the right axial region, a mass of larvae was found attached dorsal to the serratus dorsalis muscle. Tissue sections revealed a few large vesicles still in the lungs; these were surrounded by connective tissue. Budding was not noted. No larvae remained in the liver.

126th day: The lungs of a ground squirrel killed on the 126th day were macroscopically normal. Four larvae were found: 1 was embedded in muscle tissue near the aorta, dorsal to the heart; 1 was attached to the lower trachea; 1 was found free in the abdominal cavity; and 1 was in the pelvic cavity. All had long peduncles with mature scolices.

241st day: A porcupine given eggs on 2 successive days was killed on the 241st day after the first feeding. The pulmonary pleura was thickened focally, and the affected areas had numerous projecting strands of fibrinous tissue. White, consolidated areas were observed peripherally when the lungs were cut. Three mature larvae were found in the pleural cavities.

Larvae in a Naturally Infected Porcupine: Fifty-five porcupines from various localities in Alaska (see fig. 1) were autopsied to obtain information on the occurrence of the larval cestode. Only 1, collected about 95 miles northeast of Anchorage by Mr. Edward Beeman, was found to be infected. This animal, killed on January 8, 1959, was a young female weighing 12 pounds.

The pleural cavities contained 9 fully developed larvae, of which 8 were on the left side. Macroscopically, the right lung appeared normal; the lower portion of the diaphragmatic lobe of the left lung was firmly adhered along its costal surface to the parietal pleura, the basal borders of all 3 lobes having numerous fibrinous strands (fig. 10). Areas of thickened pleura, with considerable fibroblastic pro-

FIGURE 10. Lung of porcupine naturally infected with *T. twitchelli,* showing fibrinous strands along borders (dorsal view).

liferation, were evident in sections of the left lung; focal areas of lymphocytic aggregation, apparently residual inflammatory effects of the infection, were present. The interstitial capillaries were severely congested, probably an immediately *antemortem* phenomenon. Only a few aggregations of lymphocytes were present in sections of the right lung.

These larvae differed in form from any observed in experimentally infected animals. All were quite elongate, and more or less cylindrical, measuring as much as 62 mm in length (fig. 11), and each had a single scolex. Superficially they re-

sembled the mature cysticerci of *T. hydatigena* Pallas, 1766, although the vesicles were not so well developed. One was fed to a ferret, and 2 to a wolverine, in an effort to rear the adult cestode.

Non-Susceptible Animals Exposed to Infection: Attempts to infect mammals of several species were unsuccessful. Three series of red-backed voles were exposed, and one animal from each series was examined daily. Negative results

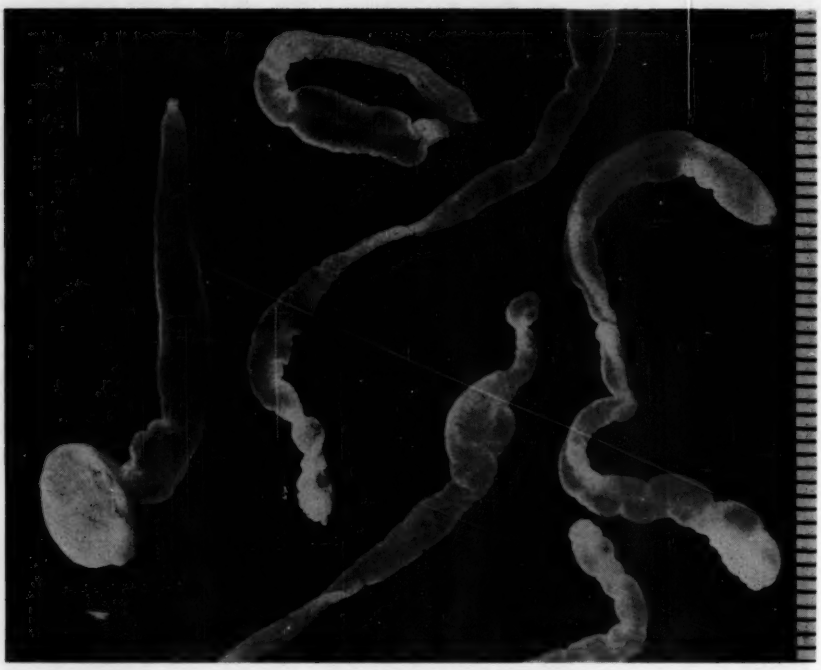


FIGURE 11. Infective larvae of *T. twitchelli* from pleural cavities of a naturally infected porcupine; scolex of one enclosed by mass of fatty tissue. (Each space of scale has value of 1 mm.)

were obtained in the first series of 9 that had received about 600 eggs each. Fifteen animals in the second series were each given about 1000 eggs. On the 4th day after exposure, one vole exhibited rapid respiration, erected pelage, and reluctance to move. At autopsy the lungs were found to be severely congested. Three others died on the 5th day after having passed blood-stained fluid from the nostrils. The lungs of 2 of these were much congested, the pleural cavities contained small quantities of free blood, and one showed petechial hemorrhages on the surface of the liver. The third animal, partially eaten by its cage-mates, was not examined. Many atelectatic alveoli and areas of microscopic emphysema, marked interstitial and limited intra-alveolar hemorrhage, thrombosis of some vessels, as well as the previously mentioned congestion, were evident in tissue sections of the lungs of the latter 3. The liver of the one contained small, scattered, granulomatous lesions in which polymorphonuclear leukocytes were predominant. The central veins were

congested, and the hepatic cells, having undergone degenerative changes, were granular in appearance in large areas. No larval cestodes were recognized in this material.

In the third series of 14 voles, each of which had received about 1000 eggs, 4 animals were severely ill on the 5th day. Two of the latter were killed for examination, and the others recovered. Tissue sections of the lungs resembled those from the previously discussed series. The animals that survived were examined only macroscopically.

The lungs of 2 hamsters killed on the 23d day after having received several hundred eggs showed 2 and 4 macroscopically visible foci, respectively, which, in tissue sections, were found to be small, granulomatous lesions with necrotic centers, each surrounded by a zone of lymphocytes with a few eosinophils. These lesions presumably represented the loci of larvae overcome by the tissue reaction of the hosts. Similar lesions, some with foreign-body giant cells, were observed in the lungs of 2 tundra voles killed on the 28th day after exposure.

A young rhesus monkey which had received several thousand eggs (6 gravid segments) manifested no clinical evidence of infection. When examined on the 126th day, the liver and lungs were grossly negative, but sections of the latter revealed some rather large areas of granulomatous reaction, consisting of necrotic foci surrounded by polymorphonuclear leukocytes, lymphocytes, a few giant cells, and limited fibroblastic proliferation. No larvae were identified.

Two white mice, each receiving several hundred eggs, were examined on the 23d day after exposure. Macroscopically, their lungs appeared normal. They had, respectively, 2 and 3 malformed larval vesicles, up to 3 mm in length, free in the pleural cavities. Sections showed a thickened pleura with a few small foci of leukocytic infiltration in the lungs, and, in the liver, small, granulomatous lesions in which it was impossible to identify any larvae. Four other white mice similarly exposed disclosed no larvae when examined on the 63d day. Nothing was found in 2 white rats which had received about 1000 eggs each.

Pathological Effects of the Larval Cestode: After entering the portal circulation, the onchospheres of *T. twitchelli* usually pass through the liver and localize in the lungs of the intermediate host. Compared with the larvae of other species of *Taenia*, the larval *T. twitchelli* is unusually pathogenic, the degree of pathogenicity manifested being proportional to the number of eggs ingested; a few larvae may cause recognizable changes in the lungs, and larger numbers can damage these organs so severely that death results. The liver is sometimes involved, but the infections so far observed in susceptible animals have not been sufficiently extensive to impair the function of this organ. In experimental animals, larvae have sometimes been observed in locations attainable only by means of the systemic circulation.

The gross pathological findings in susceptible animals have been described briefly under the section on larval development. The histopathological findings in the same animals are presented below.

The lungs of field voles on the 9th day contained many large foci of inflammatory reaction with extensive infiltration of polymorphonuclear leukocytes and lymphocytes, along with scattered eosinophils and foreign-body giant cells in some areas. Early fibroblastic proliferation was evident around some foci. Few alveoli appeared normal, and many contained erythrocytes; the focal pneumonitis was so extensive that the lungs had become essentially non-functional.

Sections from the lungs of the red squirrel on the 17th day disclosed few normal alveoli; most were either atelectatic, or no longer recognizable as such. Scattered throughout the lungs were extensive areas of pneumonitis; large areas were infiltrated with polymorphonuclear leukocytes and lymphocytes. Erythrocytes were found throughout the tissue, and all vascular channels were much congested, some thrombosed as well. The diaphragmatic lobes had extensive areas of hemorrhage. Except for a few deeply-seated vesicles, the larvae had entered the pleural cavities; some of those remaining were in part surrounded by atelectatic alveoli, apparently compressed by the migrating larvae. Others were surrounded by a zone of leukocytic infiltration comprised of both polymorphonuclear leukocytes and lymphocytes. Focally, giant cells were numerous, as were scattered concentrations of macrophages with foamy cytoplasm. The pleural surfaces showed focal concentrations of leukocytes marking points of larval penetration, aggregations which consisted of lymphocytes and macrophages. Coagulated blood often was present around the openings in the pleura.

Severe diffuse pneumonitis characterized by marked edema was evident in the lungs of the porcupine on the 13th day. Larvae were numerous and usually surrounded by zones of polymorphonuclear leukocytes. Many alveoli were filled with erythrocytes, all vascular channels were much congested, and macrophages containing hemosiderin were scattered throughout the tissue. On the 20th day (porcupine) some larvae were surrounded by dense aggregations of polymorphonuclear leukocytes, while others, apparently migrating, were in contact with atelectatic alveoli. Hemosiderin-filled macrophages were abundant, many alveoli contained erythrocytes, and a purulent exudate was evident in some areas. In the liver, the rather severe fatty metamorphosis noted probably had resulted from nutritional deficiencies during captivity. The larvae in the liver were surrounded by wide zones of acute inflammatory reaction consisting almost entirely of polymorphonuclear leukocytes. On the 24th day (porcupine), few larvae were found in the lungs, which contained extensive areas of pneumonitis with both polymorphonuclear leukocytes and lymphocytes, many atelectatic alveoli, and numerous thrombosed vessels. The fibrosed and thickened pleura was covered by a layer of acute inflammatory exudate in which polymorphonuclear leukocytes predominated.

The changes seen in sections of the lungs of the muskrat on the 60th day were compatible with the longer duration of the infection. About one-half of the total volume of the lungs was occupied by apparently immobilized larvae surrounded by connective tissue. The adjacent alveoli were atelectatic, evidently a result of continuing larval growth. Other vesicles were surrounded in part by focal concentrations of mononuclear cells. There were scattered areas of focal pneumonitis, considerable interstitial hemorrhage, and thrombosis of the smaller vessels. The pleura appeared normal.

No histopathological changes were found in the lungs of the brown lemming on the 63d day. The lungs of the muskrat that died on the 67th day were congested and showed some interstitial hemorrhage and edema, but there was no indication that *Taenia* infection was the cause of death. No tissues were preserved from the muskrat killed on the 91st day, nor from the lemming on the 94th day. Except for a few scattered aggregations of lymphocytes, the lungs were normal in the muskrat examined on the 96th day.

Sections of the lungs of the ground squirrel on the 109th day resembled in some details those from the porcupine on the 60th day. The surface of the edematous and thickened pleura was covered by an irregular layer of fibrino-purulent exudate. The inflammatory reaction was confined mainly to the pleural and subpleural areas, the latter showing extensive lymphocytic infiltration. The alveoli were characterized by atelectasis and considerable interstitial hemorrhage. The vascular channels were congested, and the bronchioles contained an amorphous eosinophilic material. Those larvae remaining in the lungs were surrounded by a zone of connective tissue adjacent to which were focal aggregations of mononuclear leukocytes.

The lungs of the porcupine on the 241st day contained areas of adenomatous proliferation, with associated atelectasis and minimal inflammation. Whether the adenomatous tumor was malignant or whether it was in any way connected with the *Taenia* infection was not determined. No lesions definitely attributable to the infection were identified.

III. The Host Relationships of *T. twitchelli*

The composition of the diet of wolverines in Alaska is largely unknown, save for reports in the literature that they feed upon the carrion of various species of ruminants. In the course of the present work, records on stomach-contents were kept. A number of the wolverines had remained in traps for some time and, consequently, their stomachs were empty. Of the 20 stomachs containing identifiable material, 12 (60 percent) held remains of moose or caribou, presumably carrion since the wolverines had been taken where carcasses of these deer were known to be available. Remains of porcupines, in 2 cases only the quills, were found in 3 (15 percent) of the stomachs. A vole, *Microtus* sp., and a ground squirrel, *Citellus undulatus* Pallas, were found in the young wolverine killed during July.

The following animals were also recorded from wolverines killed in the winter: snowshoe hare, *Lepus americanus* Erxleben; red-backed vole, *Clethrionomys rutilus* Pallas; magpie, *Pica pica* (Linnaeus); unidentified hawk; unidentified bird; and remains of fish.

The high prevalence of *T. twitchelli* in wolverines indicates that the larval stage occurs in a species of mammal eaten regularly. The finding of porcupine remains in 3 of 20 stomachs indicates the importance of the porcupine as a source of food. Porcupines, distributed widely and at times locally abundant in Alaska, would seem to be particularly vulnerable to predation by wolverines during the winter months, when they remain in or near the trees where they feed. During this time, few species of smaller mammals are available, for ground squirrels hibernate and microtine rodents are almost entirely subnivean in their activities. Among the latter, only red-backed voles (shown to be resistant to infection) move about rather freely on the surface of the snow. Red squirrels, *Tamiasciurus hudsonicus* (Erxleben), and flying squirrels, *Glaucomys sabrinus* (Shaw), remain active, but neither would be frequently captured by wolverines, nor has *T. twitchelli* been found in the large numbers of these and other common small rodents examined in Alaska. The porcupine appears to be the essential intermediate host of this cestode.

The adult *T. twitchelli* is probably host specific. Depending upon geographic location, the biotope of the wolverine may be shared in Alaska by any or all of the following mustelids: marten, *Martes americana* (Turton); ermine, *Mustela erminea* Linnaeus; least weasel, *M. nivalis* Linnaeus (cf Reichstein, 1957); mink, *M. vison* Schreber; and otter, *Lutra canadensis* (Schreber). *T. twitchelli* has never been recorded from any of these, nor do their cestodes (*T. mustelae* and *T. martis*) parasitize the wolverine. None of the aforementioned species, however, is known to prey upon porcupines, so the apparent host specificity may be ecological rather than physiological. Another mustelid, the fisher, *Martes pennanti* (Erxleben), inhabits the boreal forest as far north as southern Yukon Territory and also preys upon porcupines (Quick, 1953; Hamilton and Cook, 1955). Possibly *T. twitchelli* will be found also in the fisher when its helminth parasites are better known.

A few attempts to infect other species of mustelids experimentally have been unsuccessful or inconclusive. A ferret, *Mustela putorius* Linnaeus, received a 126-day-old larva with 18 scolices from an experimentally infected ground squirrel, but daily fecal examinations disclosed no eggs, and no cestodes were found when the animal was autopsied 55 days later. A second ferret received a larva with 10 scolices from the same source and likewise was uninfected when autopsied. A third ferret, given a single larva from the naturally infected porcupine, harbored no cestodes when it was killed after 22 days. A wild-trapped mink received a 241-day-old larva with 10 scolices from an experimentally infected porcupine. This mink died on the 7th day following exposure. Since it was not immediately examined, the 2 immature cestodes found, both about 10 mm long, were unidentifiable due to the effects of post-mortem degeneration of the intestine.

The adult cestode was reared only once under controlled conditions. One wolverine, a captive for about 2 years and known to be free of infection, was fed 2 larvae from a naturally infected porcupine. For 7 weeks before the experiment was terminated, fecal smears were examined daily for *Taenia* eggs with negative results. At autopsy, on the 66th day after exposure, 2 cestodes were found attached

to the mucosa of the small intestine 1020 mm posterior to the pylorus. These measured 74 mm (106 segments) and 90 mm (113 segments) in length, respectively. The uterus was well developed in the terminal segments of both, but only the last segment of the larger strobila contained a few eggs. Although the time required for *T. twitchelli* to produce infective eggs was not determined, it probably is not less than 75 days.

DISCUSSION

Seven species of helminths have been recorded from wolverines in Alaska; viz, *Alaria* sp., *Mesocestoides kirbyi, Taenia twitchelli, Trichinella spiralis, Malineus patens, Ascaris devosi,* and *Physaloptera torquata.* Only one additional species has been reported from this mammal in North America: Price (1930) identified *Soboliphyme baturini* Petrov, 1930, from a wolverine killed in Montana, and Bezdek (1942) cited a record from a wolverine in Canada. I have identified *S. baturini* from other species of mustelids in Alaska (mink and marten), and suspect that it occurs here in wolverines also.

Several workers have mentioned the occurrence of *Pseudamphistomum truncatum* (Rudolphi, 1819) in wolverines in Eurasia (cf Price, 1932; Skriabin, 1950), and Hilgendorf and Paulicki (1870) reported *Opisthorchis felineus* (Rivolta, 1884). Erhardt (1935) believed that the latter infection had been acquired in the Hamburg Zoological Gardens where the animal died, rather than in northern Europe where it was captured. No cestodes have been reported from wolverines in Eurasia, but Spasskii et al (1952) and Machul'skii (1953) found 5 species of nematodes; viz, *Capillaria putorii* (Rudolphi, 1819), *Molineus patens, Ascaris columnaris, Physaloptera sibirica,* and *Soboliphyme baturini.* Some older records of uncertain status also exist (cf Stiles and Baker, 1935), but these have not been considered here.

Three of the helminths (*T. spiralis, M. patens,* and *S. baturini*) are now known to be holarctic in distribution. To these may be added *Ascaris devosi,* if later studies establish that the latter has been erroneously identified as *A. columnaris* in Eurasian mustelids. Another nematode, *Capillaria putorii,* although never validly recorded in North America, possibly is present (Read, 1949). Although there now is no contrary evidence, the existence of 2 species of *Physaloptera* in mustelids in boreal regions seems questionable.

The trematodes *Pseudamphistomum truncatum* and *Opisthorchis felineus* are found only in Eurasia, while the cestodes, *Mesocestoides kirbyi* and *Taenia twitchelli,* are known only from North America. The adult *T. twitchelli* is evidently host specific for the wolverine, a holarctic species. On the hypothesis that the larva also is host specific, at least ecologically, it follows that the cestode is restricted distributionally to North America. Erethizontid rodents first appeared in South America during the Oligocene, and *Erethizon* entered North America during Pliocene time over the Panama land bridge. Apparently, however, it had not extended its range far enough in North America to make its way into Eurasia during Pleistocene time (Simpson, 1945; Burt, 1958). It appears, then, that the host relationships of this cestode evolved in North America. If *T. twitchelli* were introduced into Eurasia, it seems doubtful that any mammalian species there would be both susceptible to infection by the larval cestode and preyed upon by wolverines

to the degree necessary to ensure the perpetuation of the species.

T. twitchelli is one of the few species of *Taenia* known to reproduce by means of exogenous budding in the larval stage. The hexacanth embryos characteristically localize in the lungs of the intermediate host, and after about 2 weeks the larvae appear as translucent, more or less ovoid bodies measuring up to about 1.5 mm in length. At this time, they consist essentially of a thin cuticle enclosing delicate, reticular tissue, and closely resemble the larvae of other species of *Taenia* (e.g., *T. pisiformis* Bloch, 1780) at a comparable stage of development.

Intrapulmonary migration of the larvae evidently begins in porcupines at least as early as the 11th or 12th day following exposure, after which time each larva is found at the end of a leukocyte-filled tunnel with its anterior end in contact with ostensibly normal host tissue. The larvae, presumably, secrete a proteolytic enzyme enabling them to penetrate the tissue. From about the 16th to the 25th day, they break through the pulmonary pleura, escaping into the pleural cavities where their subsequent development takes place.

Since the emigrating larvae, when numerous, severely injure the lungs of the host, the onset of acute illness in some of the massively infected animals was taken to indicate the time of emergence of the larvae from the lungs. In those animals that died within about 24 hours after the first signs of acute respiratory disorder, the pleural cavities contained great numbers of larvae, while few remained within the lungs. The uniform size and state of development of those in the pleural cavities further indicate that the emigration of the larvae takes place over a relatively short period of time. In some animals, their emergence was apparently delayed, or perhaps prevented, by the severe inflammatory reaction they had evoked. For example, the pulmonary pleura was much thickened and fibrosed in a porcupine that died on the 24th day after exposure, and the majority of larvae had not yet emerged. Larvae were found also in the lungs of other hosts that had survived massive infections for a relatively long time, but none was found in the lungs of animals that had received light infections.

The maximum size normally attained by the larvae in the lungs of the host was not determined, since no animals were autopsied just at the time the larvae were beginning to emerge, but they evidently develop rapidly after they reach the pleural cavities. Ordinarily, the larvae do not become vesicular until after they enter the pleural cavities, although the change to this form eventually occurs even in those which never migrate from the lungs. The youngest larvae found in the pleural cavities were vesicular and had already produced buds. Irregularly spaced aggregations of cells in the vesicle wall indicate the sites of budding, and the buds are produced simply as evaginations of the vesicle wall. In some cases, invagination around the cellular aggregations occurs, but it is usually not seen until the buds are well defined; typically, such invagination is apical and results in the enclosure of the primordium of the scolex. Development is not uniform, and a single vesicle may have primordia of scolices in various stages of formation as well as buds of different sizes.

Soon after their appearance, the buds contain a persistent, delicate reticulum, usually represented in the mature larva by only a few strands of tissue. As the buds lengthen and become more or less pedunculate, the vesicle from which they originated is often largely obliterated and the scolex-bearing peduncles appear to

be attached at a common point. The number of peduncles on mature larvae was found to range from 1 to 33, with an average of about 10. Secondary buds are sometimes produced from the walls of earlier buds, and 2 scolices are sometimes found at the apex of a single peduncle. Endogenous budding from the wall of the primary vesicle is not uncommon. Although permanently enclosed by either the primary vesicle or by another bud into which it protrudes, the peduncles arising in this manner developed normally and produced fully formed scolices. Some pleomorphism in mature larvae appears to be related to host species. Larvae from lemmings, and sometimes from ground squirrels, had a somewhat spherical shape, with protruding short stalks, while those from muskrats and porcupines exhibited little trace of the original vesicle and possessed long peduncles. There is also a direct relationship between host size and the size of the larvae.

Secondary vesicles are sometimes produced by exogenous proliferation, beginning as evaginations of the wall of the primary vesicle and at first resembling typical buds. They then become constricted at the base, assume a discrete form, and usually become detached. Such secondary vesicles may show no signs of the primordia of scolices before becoming detached, but they later produce buds and apparently develop in the same way as the primary vesicles. The earliest formation of secondary vesicles observed was in a massively infected muskrat that died on the 60th day. Whether this process occurs regularly in the larval *T. twitchelli* is not known; however, mature larvae were also found with secondary vesicles still attached. In some cases, at least, small larvae with single scolices must have originated in this manner. Among other species of *Taenia,* only the cysticercus of *T. crassiceps* (Zeder, 1800) is known normally to produce secondary vesicles by means of exogenous proliferation.

The development of the mature larva from the primary vesicle of *T. twitchelli* appears to follow about the same pattern as described by Freeman (1956) for the multiscolex-type larva of *T. mustelae* Gmelin, 1790. In fact, some of the smaller larvae of *T. twitchelli,* such as those from lemmings, probably would be macroscopically indistinguishable from those of *T. mustelae.* The typical, mature larva of *T. twitchelli,* such as is usually found in porcupines, seems to resemble most closely the larva of *T. endothoracicus* Kirshenblat, 1948 (*vide* Dollfus, 1956; fig. 3). The larvae of the latter occur in various species of rodents and, according to Dubnitskii (1952), the adult cestode is a parasite of the red fox, *Vulpes vulpes* Linnaeus. A rather similar form, *Taenia* sp. indet., was described by Voge (1954) from the body cavity of Peruvian rodents.

Certain genera of the *Taenia*-group, all of which have morphologically similar adults, have been established primarily on the basis of larval characteristics. As various species become better known, however, it becomes increasingly apparent that the extent of morphological diversity, both intraspecific and interspecific, in their larval stages is often so great as to preclude their allocation to the existing genera. In my opinion, the establishment of taeniid genera based on the morphological characteristics of the larval stage is untenable, and it seems best to accept the genus *Taenia* in its widest application until such time that a critical revision of the group may be practicable. Although *T. twitchelli* was allocated to the genus *Multiceps* Goeze, 1782, by Clapham (1942), it is considered more appropriate to retain it in the genus *Taenia,* to which it was originally assigned by Schwartz (1924).

SUMMARY

Seven species of helminths were collected from 80 wolverines autopsied in Alaska. Four species, viz, *Alaria* sp., *Mesocestoides kirbyi* Chandler, 1944, *Ascaris devosi* Sprent, 1952, and *Physaloptera torquata* Leidy, 1856, are recorded for the first time from this host, and a fifth, *Molineus patens* (Dujardin, 1845), for the first time from the wolverine in North America.

The life cycle of *Taenia twitchelli* Schwartz, 1924, a cestode apparently host specific for the wolverine, was completed experimentally, and the essential natural intermediate host is considered to be the porcupine, *Erethizon dorsatum*. Observations on host relationships and on the development and pathogenicity of the larval cestode are described. The original description of *T. twitchelli* has been supplemented and the taxonomic status of this species discussed.

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ADDENDUM

The writer has recently autopsied 28 additional wolverines taken in the Anaktuvuk Pass region, central Brooks Range, during the winter of 1958–59. The numbers of infected animals were as follows: *Taenia twitchelli,* 25; *Ascaris,* 11; *Physaloptera,* 1. No helminths were found in 2 animals.

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THE USE OF METABOLIC ANTIGENS IN THE FLOCCULATION TESTS FOR THE SEROLOGIC DIAGNOSIS OF TRICHINOSIS

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In an earlier paper from this laboratory, it was reported that an acid soluble protein fraction of *Trichinella* larvae when used as an antigen produced a flocculation test which was easy to read and to interpret (Sadun and Norman, 1955). Investigations with experimentally infected rabbits indicated that a metabolic antigen obtained from the excretions and secretions of *Trichinella* larvae conferred to the flocculation test a somewhat greater sensitivity than the purified larval somatic antigen (Sadun and Norman, 1956). The present series of experiments was designed to test whether the metabolic antigen can be used effectively in the rapid flocculation test for the diagnosis of trichinosis in humans and lower animals. In order to obtain some evidence as to whether the two antigens are qualitatively different or whether they differ only in the relative proportion of common antigens, an attempt was made to absorb antibodies from the serum of rabbits with each of the two antigens.

MATERIALS AND METHODS

The metabolic antigen and the purified somatic antigen were prepared according to the published method (Sadun and Norman, 1956). The flocculation test was performed with strict adherence to the method described by Bozicevich (Bozicevich, et al, 1951).

Rats, rabbits, and hogs were used as experimental animals. The rats used were young adults weighing between 130 and 150 g. After they had each been inoculated intragastrically with approximately 3,000 washed larvae, they provided the fresh larvae used in preparing the antigens and for infecting the rabbits and hogs. At least 30 days after the rats were infected, the larvae were separated from the muscles by artificial digestion in a pepsin-hydrochloric acid mixture. The rabbits used in this study were adults varying in weight from 2,000 to 4,000 grams at the beginning of the experiment. Some were given intragastrically variable numbers of washed larvae. Others were inoculated intramuscularly with either the somatic antigen or the metabolic antigen in aluminum hydroxide (Dorin, 1946). Consistently high levels of reactivity were obtained 2 to 3 weeks following a single inoculation even when relatively small amounts of antigen were used. The hogs in this series were 4 to 7 weeks old at the time they were inoculated intragastrically with variable numbers of *Trichinella* larvae. They had been reared under controlled experimental conditions at the Alabama Polytechnic Institute, School of Veterinary Medicine.

The rabbits were bled from the marginal ear vein, and occasionally from the heart when large amounts of serum were needed. The hogs were bled from the vena cava. In addition, sera from 163 wild animals trapped in Southern Georgia were examined in an attempt to obtain some indirect evidence as to the specificity of the flocculation test with either of the two antigens.

A total of 1,086 serum specimens from humans suspected of being infected with *Trichinella* were tested with both antigens. Some of the human sera had been treated with merthiolate before being shipped to the CDC laboratories. Others arrived with obvious signs of bacterial contamination. However, neither merthiolate in the amounts used for serum preservation nor the bacterial contamination seemed to interfere with the flocculation reaction with either antigen tested. Clinical histories, including laboratory findings, symptoms, and tentative diagnoses were available for some of the patients whose sera were tested. Information proving or strongly suggesting infection with *Trichinella spiralis* was obtained on 50 patients.

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In order to determine whether the purified somatic antigen and the metabolic antigen stimulate distinct humoral factors, absorption of the antibodies against these antigens was attempted. Rabbit sera were mixed with serial dilutions of the respective antigens and incubated for variable amounts of time at 56°, 37°, and 4° C. Precipitates which were produced were removed by centrifugation and the supernatant solutions were retested by the flocculation reaction to determine the amounts of antibodies present.

The total nitrogen in the two antigens was determined by the microkjeldahl method, and the total protein in the somatic antigen by biuret reaction.

RESULTS

1. Flocculation Test with Human Sera. Of the 1,086 serum specimens from patients suspected of having become infected with trichinosis, 810 gave negative reactions and 216 gave positive reactions with both antigens (table I). Of the 60 sera

TABLE I. *Comparison of metabolic and purified somatic antigen in the flocculation test on 1086 sera from humans suspected of having trichinosis.*

Metabolic antigen	Purified somatic antigen											Total
	Neg.	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	
Neg.	*810*	5	3									818
1:5	34	6	4	1			1					46
1:10	11	13	4		1							29
1:20	7	5	23	*11*	2							48
1:40		1	3	14	4	2						24
1:80			3	12	15	4	5	2				41
1:160				1	6	17	7		1			32
1:320					1	2	6	*8*	1			18
1:640							4	9	4			17
1:1280								1	3	4	1	9
1:2560										1	*1*	2
1:5120											2	2
Total	862	30	40	39	29	25	23	20	9	5	4	

giving contrasting reactions with the two antigens, 52 were positive with the metabolic, and 8 were positive with the somatic antigen. The differences occurred exclusively among sera which were positive with the other antigen at a low dilution (1:20 or less). The difference between those that were positive with the metabolic antigen and those that were positive with the somatic antigen alone is highly significant (t equals 5.55). One hundred ninety-four sera gave a higher titer with the metabolic, and 29 sera gave a higher titer with the somatic antigen. This difference is also highly significant (t equals 10.99). On the basis of detailed clinical histories and other laboratory tests such as muscle biopsy, 50 sera were selected as coming from individuals with proved trichinosis. The results of a comparison of the reactions obtained with the flocculation test by using either of the 2 antigens on these 50 sera are summarized in table II. Of 50 specimens, 3 were negative, and 45 were

TABLE II. *Comparison of metabolic and purified somatic antigens in the flocculation test on 50 sera from humans with proven or highly suggestive trichinosis.*

Metabolic antigen	Purified somatic antigen											Total
	Neg.	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	
Neg.	*3*		1									4
1:5		*1*	1	1								3
1:10		2	*1*									3
1:20	1		1	*3*	1							6
1:40			1	3	2							6
1:80				2	2	*1*		1				6
1:160					1	3	3					7
1:320							3	*3*				6
1:640								3				3
1:1280								1		*3*	1	5
1:2560											*1*	1
Total	4	3	5	9	6	4	6	8	0	3	2	

positive with both antigens. One serum specimen gave positive reactions with the metabolic antigen alone and the other with the somatic antigen alone. Twenty-three sera gave higher titers with the metabolic antigen, and 6 sera gave higher titers with the somatic antigen. This difference is significant at the 5 percent level (t equals 2.97).

2. Flocculation Test with Hog Sera. In this experiment, 108 sera were collected from uninfected hogs and from litter mates which were experimentally infected with single doses of *T. spiralis* varying from 5,000 to 50,000 larvae each. Since most of the specimens were obtained from uninfected hogs or from infected litter mates during the first few weeks following inoculation, a large percentage of negatives was to be expected in this group. As indicated in table III, 76 sera were

TABLE III. *Comparison of metabolic and purified somatic antigens in the flocculation test on 108 sera from swine experimentally infected with* Trichinella spiralis.

Metabolic antigen	Purified somatic antigen						Total
	Neg.	1:5	1:10	1:20	1:40	1:80	
Neg.	76	2	1	1			80
1:5	3	1	2	1			7
1:10	4	2	2			1	9
1:20		2		4	2	1	9
1:40				1	1	1	3
Total	83	7	5	7	3	3	

negative, and 21 were positive with both antigens. Of the 11 giving discrepant results, 7 were positive with the metabolic and 4 with the somatic antigen. These differences occurred exclusively among sera which were positive with the other antigen at a dilution of 1:20 or less. As indicated in table III, no consistent differences were observed with either of the 2 antigens, and no significant differences in the sensitivity of the 2 antigens could be observed.

3. Flocculation Tests with Sera from Wild Animals. In order to obtain some indirect evidence as to the specificity of the flocculation test with either of the 2 antigens, sera from 163 wild animals trapped in southern Georgia were tested. Necropsies performed on a few of these animals revealed rather heavy infections with various cestodes, trematodes, and nematodes. Of the 163 sera, 31 came from raccoons, 21 from opossums, 4 from cottontail rabbits, 21 from foxes (grey and red), 14 from skunks (striped and spotted), 11 from bobcats, 9 from fox squirrels, 25 from moles, and 26 from wild rats. One hundred fifty-six sera gave negative reactions with both antigens. Five sera (4 from wild rats and 1 from a raccoon) were positive with both antigens at a titer of 1:5. One (from a wild rat) was positive at a 1:5 titer with the somatic antigen and negative with the metabolic antigen while 1 (raccoon) was positive at a 1:5 titer with the metabolic and negative with the somatic antigens.

4. Absorption of Homologous Antibodies from Immune Serum of Rabbits. Several lots of metabolic antigen averaged 0.2 mg N per ml. The medium in which the larvae grew averaged 0.16 mg N per ml. The somatic antigen solution contained 0.12 mg N per ml by microKjeldahl and 0.725 mg protein by biuret. For absorption studies, the somatic antigen was diluted so as to give a protein concentration similar to that of the metabolic antigen. Preliminary experiments indicated that the amount of metabolic or somatic antigens needed to absorb completely the precipitable antibodies to either of the 2 antigens in the immune serum varied from one specimen to another. Therefore, different antigenic concentrations were used

with each serum. Also, preliminary tests indicated that no differences in the reactions took place whether the serum-antigen mixtures were refrigerated at 4° C overnight or incubated at 37° C for 60 minutes or in a water bath at 56° C for 20 minutes. In view of these observations, overnight refrigeration at 4° C was used for convenience in the experimental set up. Comparable results were obtained when the solutions were left at 4° C in the refrigerator for longer periods of time, thus indicating that overnight refrigeration was sufficiently long to absorb all antibodies which could be absorbed by this method.

Serial dilutions in the amount of 0.3 ml of the antigenic solutions were added to 0.3 ml of serum in 10 by 75 mm test tubes. The solutions were mixed and refrigerated at 4° C overnight. Borated merthiolate to the concentration of 1 : 10,000 had been added to all reagents to inhibit bacterial growth during incubation. The results have been summarized in table IV. The three sera from group I were obtained from

TABLE IV. *Results of serum absorption with metabolic and somatic antigens.*

Group	Antiserum	Results of flocculation test (Reciprocal Titers)					
		Before absorption		After absorption with metabolic antigen		After absorption with somatic antigen	
		Metabolic antigen	Somatic antigen	Metabolic antigen	Somatic antigen	Metabolic antigen	Somatic antigen
I	1	—	20	—	5	—	—
	2	5	20	—	10	—	—
	3	40	20	—	5	20	—
II	4	5	—	—	—	—	—
	5	20	—	—	—	20	—
	6	80	10	10	—	80	—
III	7	5	180	—	160	—	—
	8	5	160	—	160	—	—
	9	160	160	—	20	40	10
	10	10	40	—	10	—	—
IV	11	160	80	—	10	40	5
	12	320	180	80	160	40	5
	13	320	160	5	10	160	40
	14	80	40	20	10	20	—
	15	80	20	—	—	10	—

rabbits which had been artificially immunized with somatic antigens and the three sera from group II from rabbits artificially immunized with metabolic antigen. The sera from group III were obtained from rabbits which had been inoculated intramuscularly and intravenously with larvae. The sera of group IV were obtained from rabbits which had been fed intragastrically with variable numbers of washed *Trichina* larvae. A perusal of table IV indicates that antibodies were absorbed by the homologous antigens in greater proportions than by the heterologous antigens. This absorption, however, also produced a considerable lowering in titer with the heterologous antigen.

DISCUSSION AND SUMMARY

The data presented reveal that the flocculation test, with both antigens, gave consistently reliable results with sera from humans, hogs, and various wild mammals. Very similar results were obtained with both antigens in all animals.

The metabolic antigen seemed to confer to the flocculation test a greater sensitivity in testing human sera. This is in agreement with observations reported in rabbits (Sadun and Norman, 1956). On the other hand, this greater sensitivity was not observed with sera of experimentally infected hogs.

The high degree of sensitivity of this test was indicated by the results obtained

from sera of humans with proven or highly suggestive trichinosis. These results are in agreement with and extend those reported by Norman and her co-workers (Norman, et al, 1956). Only indirect information is available on the specificity of this test with either of the 2 antigens. The fact that negative results were obtained with most of the sera from wild animals in spite of their being heavily infected with various helminths added to the information obtained with laboratory raised hogs and rabbits (Sadun and Norman, 1956) suggests that both antigens are rather highly specific for trichinosis. Whether or not the apparent increased sensitivity of the metabolic antigen corresponds to a lower specificity cannot be determined on the basis of the present findings.

The results on the experiments in which absorption of serum antibodies by metabolic and somatic antigens was attempted do not allow us to state with any degree of certainty whether or not the two antigens are qualitatively different. Obviously, a certain degree of cross-reaction occurs. However, the greater degree of absorption of antibodies obtained with the homologous antigen in every group of sera suggests that specific reacting fractions may be present in the two antigens.

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RESEARCH NOTE

ANOTHER SPECIES OF MICROPHALLUS WARD, 1901, FROM THE PINK SHRIMP, *PENAEUS DUORARUM* BURKENROAD.

Sogandares-Bernal and Hutton (J. Parasit. **45**: 250, 266) reported the metacercarial stage of a species of *Microphallus* Ward, 1901, from the pink shrimp. Recently, while dissecting metacercariae of this species from a juvenile pink shrimp collected near Ozona, Florida, one of us (B.E.) found a single live metacercaria of a second species of *Microphallus*. This specimen was found along with numerous live specimens of the previously reported *Microphallus*.

The pink shrimp may be an accidental host for this second species, since previously we have not found it in several thousand specimens of this crustacean collected from the east coast of Florida and the Gulf of Mexico. Figure 1 shows the encysted larva of the two species of *Microphallus*. The second species differs from the first one in the following ways: (1) the cyst is 3 to 4 times larger; (2) the acetabulum is approximately twice the size of the oral sucker, whereas, in the first species the suckers are approximately the same size; and (3) the genital pore is immediately sinistral to the acetabulum at its anterior border, whereas, in the first species the genital pore is immediately sinistral to acetabulum at its posterior border or middle.—ROBERT F. HUTTON, FRANKLIN SOGANDARES-BERNAL AND BONNIE ELDRED, *Florida State Board of Conservation Marine Laboratory, Maritime Base, Bayboro Harbor, St. Petersburg, Florida.* (Contribution No. 27)

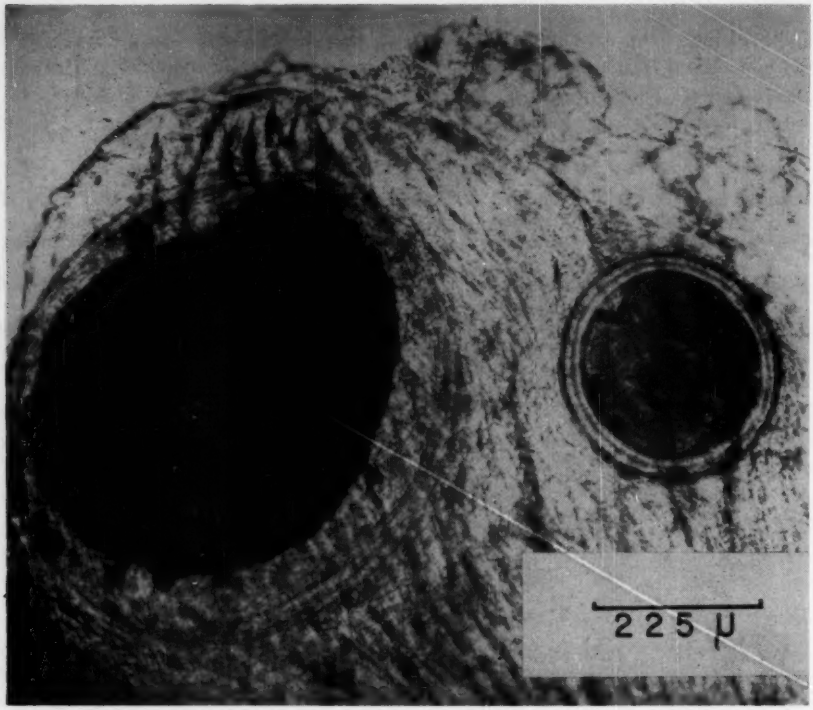

FIGURE 1. The metacercarial stage of two species of *Microphallus* in the muscle tissue of the pink shrimp.

LACTIC DEHYDROGENASE IN *TRICHOMONAS VAGINALIS*

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A large body of information is developing on the physiology of various trichomonad parasites of man and animals (Ryley, 1955; Doran, 1957; Read, 1957; Ninomiya and Suzuoki, 1952). In the hope of providing basic explanations for the physiological data, a study of the enzyme systems of these parasites is in progress in this laboratory. An attempt is being made to characterize the enzymes so that they may be compared with corresponding enzymes from other organisms and thus contribute to the development of comparative biochemistry.

Kupferberg et al (1953) showed that lactic acid accumulates in cultures of *Trichomonas vaginalis*. Homogenates of the organism can utilize lactic acid, as shown by oxygen uptake experiments in the presence of methylene blue (Witschafter and Jahn, 1956). DPN is required and, therefore, the enzyme resembles lactic dehydrogenase of muscle rather than that of yeast (Bach, Dixon, and Zerfas, 1946). The reduction of methylene blue in the oxygen uptake experiment above depends upon another enzyme, DPN oxidase, which is undoubtedly present in the homogenate (Rafter and Colowick, 1955). Our experiments with this enzyme will be reported later. The present paper describes a partially purified lactic dehydrogenase found in homogenates of the organism.

MATERIALS AND METHODS

Strain R of *T. vaginalis* was isolated, cultivated, and harvested as previously described (Baernstein, 1955). Wet weights of the harvests varied from 2.5 to 4.0 grams.

The washed organisms were transferred from the centrifuge tube to the cup of a 9-kilocycle sonic oscillator with 15 ml 0.2 M phosphate buffer, pH 7.5. Temperature during sonication was maintained at 2° C. Fifteen minutes was found to be adequate for rupture of most of the cells. Aliquots of the homogenate were taken for activity determination and the remainder transferred to a centrifuge tube and spun at 27,000g for 5 minutes at 2° C. The supernate was poured off into a test tube and stored at 2° C or frozen. The sediment was weighed and examined microscopically. Whole cells were rarely seen. The weight varied from 10–59 percent of the original wet weight.

Protein of the homogenate was determined by the method of Lowry et al (1951). Activity of lactic dehydrogenase was determined by measuring changes in optical density at 340 millimicrons with a Beckman DU spectrophotometer and Corex cells, 1.0 cm light path. One unit activity changed the density 0.01 per minute in a total volume of 3.0 ml. Specific activity was expressed in units per mg protein.

DPNH, DPN, and TPN were better than 90% pure as purchased. Substrates, inhibitors, and activators were high grade commercial preparations. (The following abbreviations are used in this paper; DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; BAL, 2, 3-dimercapto-1-propanol; tris, tris (hydroxy methyl) aminomethane.)

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RESULTS

Linearity and reversibility. Centrifuged homogenates of *T. vaginalis* oxidize lactate and reduce pyruvate in the presence of DPN and DPNH, respectively. Figure 1 shows the relative rates of these reactions and the nearly linear course during

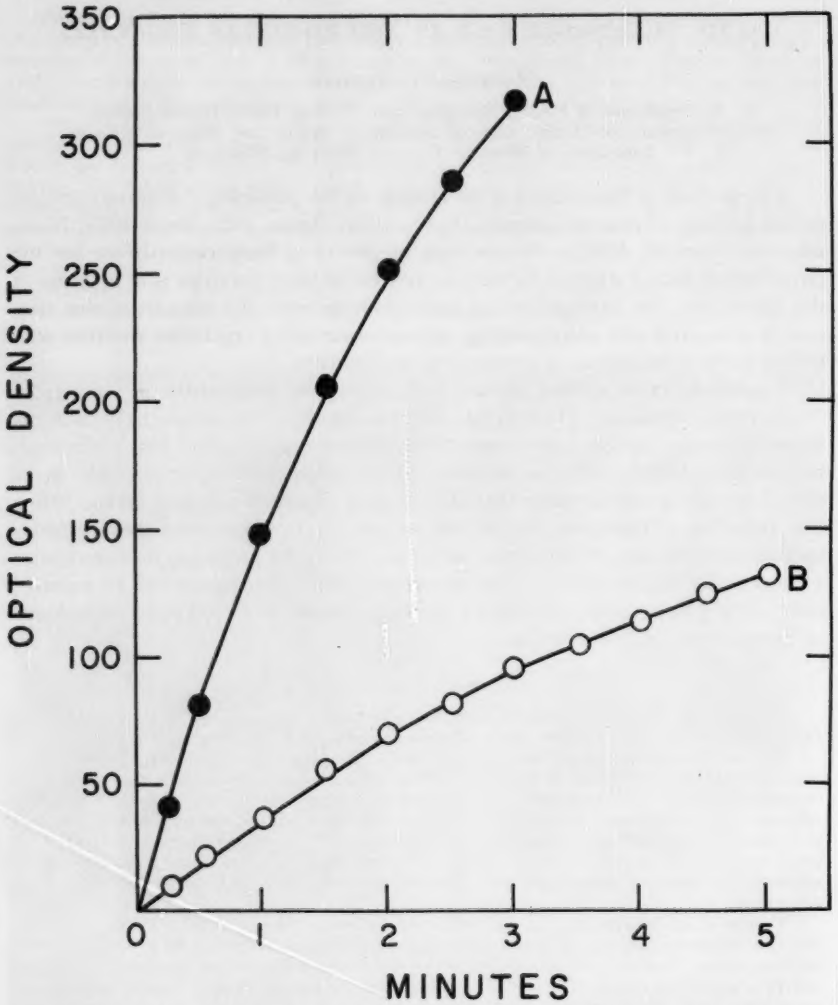

FIGURE 1. Linearity and reversibility. *Curve A.* Pyruvate reduction. Complete system: 1.6 ml DPNH solution (0.2 micromole/ml) in 0.15 M pyrophosphate buffer, pH 9.0; 0.4 ml 0.1 M sodium pyruvate; water to make 2.9 ml. Reaction started by adding 0.1 ml centrifuged homogenate diluted 1:5 and containing 0.23 mg protein. *Curve B.* Lactate oxidation. Complete system: 1.6 ml pyrophosphate buffer; 0.4 ml 0.1 M lithium lactate; 0.4 ml DPN (2 micromole per ml 90 percent pure); water to make 2.9 ml. Reaction started as above.

the first 3 minutes after starting the reaction by adding the homogenate. The re-

duction of pyruvate is the preferred reaction, because it is faster and requires no trapping agent for the products.

Proportionality between rate of oxidation of DPNH and concentration of lactic dehydrogenase is obtained when the density change is less than 0.040 per 30 seconds.

Chemical balance. Limiting amounts of pyruvate were added to mixtures of buffer, DPNH, and enzyme. The decreases in density at 340 millimicrons were determined during the course of approximately 1 hour, when the reaction had reached equilibrium. The entire contents of the cells were then treated with copper sulfate and lime in preparation for lactic acid determination (Barker and Summerson, 1941). The results in table I show a satisfactory agreement among 3 components of the reaction:

$$\text{Lactate} + \text{DPN} \rightleftarrows \text{Pyruvate} + \text{DPNH} + \text{H}^+$$

Optimum pH. Different amounts of acid or alkali were added to a series of mixtures (table I) instead of water and activities determined as described earlier. The

TABLE I. *Chemical balance.* (Mixtures contained 0.2 M phosphate, pH 7.5, 1.0 ml; DPNH 2 micromoles per ml, 0.2 ml; enzyme was a fraction of homogenate precipitated between 50 and 75 percent saturated ammonium sulfate and contained 0.76 mg protein per ml, 0.04 ml; water to make final volume 3.0 ml. Reaction started by adding 0.0 to 0.25 ml pyruvate 0.001 M. Original density corrected for slight differences in volume before addition of pyruvate. A control mixture without pyruvate served to measure endogenous activity which was subtracted from total change of density and of lactate.)

Pyruvate added (micromoles)	DPNH oxidized (micromoles)	Lactate formed (micromoles)
0.05	0.04	0.04
0.10	0.10	0.13
0.15	0.14	0.15
0.20	0.19	0.19
0.25	0.23	0.25

pH of each mixture was then determined by glass electrode. Maximum activity for pyruvate reduction was found to be at pH 7.6. At pH 7.0 and 8.5 activity was 75 percent of maximum.

Optimum DPNH concentration. The rates of reaction were determined in the presence of excess pyruvate and different amounts of DPNH. DPNH was standardized spectrophotometrically using the extinction coefficient 6.3×10^6 cm^2 per mole (Colowick et al, 1951). Maximum velocity was obtained with 11×10^{-5} M DPNH and half-maximum velocity with 1.7×10^{-5} M (K_m fig. 2). These values agree with those found for lactic dehydrogenase of mammalian heart, muscle, and *Schistosoma mansoni* (Meister, 1950; Nielands, 1952; Mansour and Bueding, 1953).

Optimum pyruvate concentration. The rates of reaction were determined in the presence of excess DPNH and different amounts of pyruvate (fig. 3). Maximum velocity was found at 1×10^{-3} M pyruvate and half-maximum velocity at 2×10^{-4} M. These values are similar to those found for lactic dehydrogenase in other organisms.

Inhibition and reversal. Vestling et al (1956) found that liver lactic dehydrogenase showed no inhibition with iodoacetate and no sulfhydryl groups when titrated in aqueous solution. The enzyme was inhibited with para-mecuribenzoate and showed 10 – SH groups per mole when titrated in 50 percent alcohol. Glutathione reversed the inhibition with para-mercuribenzoate. Others have noted the presence of relatively unreactive – SH groups in enzymes.

T. vaginalis lactic dehydrogenase shows similar behavior (table II). Iodoacetamide, iodoacetate, and arsenite did not inhibit even when incubated with the enzyme

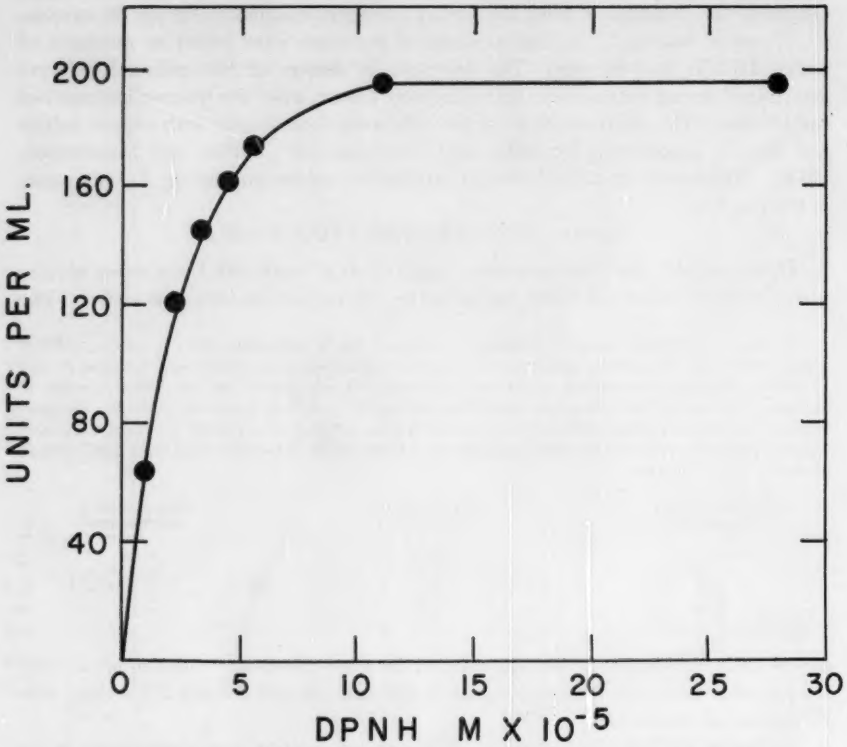

FIGURE 2. Optimum DPNH concentration. Mixtures contained 0.1 M Tris buffer pH 7.35, 1.0 ml; DPNH solution 2 micromoles per ml, 0.02 to 0.2 ml; 0.1 M pyruvate, 0.2 ml; water to make 2.98 ml; reaction started by adding 0.02 ml dilute, centrifuged homogenate containing 0.18 mg protein per ml.

for 30 minutes. Para-mercuribenzoate inhibited completely under the same conditions. Even 3 minutes were sufficient for nearly complete inhibition. Cysteine at 10 times the concentration of para-mercuribenzoate reversed the inhibition considerably, but BAL at equal concentration reversed it completely. These experiments indicate that lactic dehydrogenase of *T. vaginalis* is dependent upon – SH groups which are less reactive than those in other enzymes such as triose phosphate dehydrogenase (Ninomiya and Suzuoki, 1952).

Specificity of substrate and coenzyme. Figure 1 shows the rate of reduction of DPN by DL lactate. When this experiment was repeated using the separated isomers, only $L^{(+)}$ lactate was oxidized; $D^{(-)}$ lactate was not attacked.

TPN gave only 16 percent of the rate of oxidation of $L^{(+)}$ lactate given by DPN. When both coenzymes were present in equimolar concentration, the rate was 112 percent of the rate of DPN alone oxidizing DL lactate.

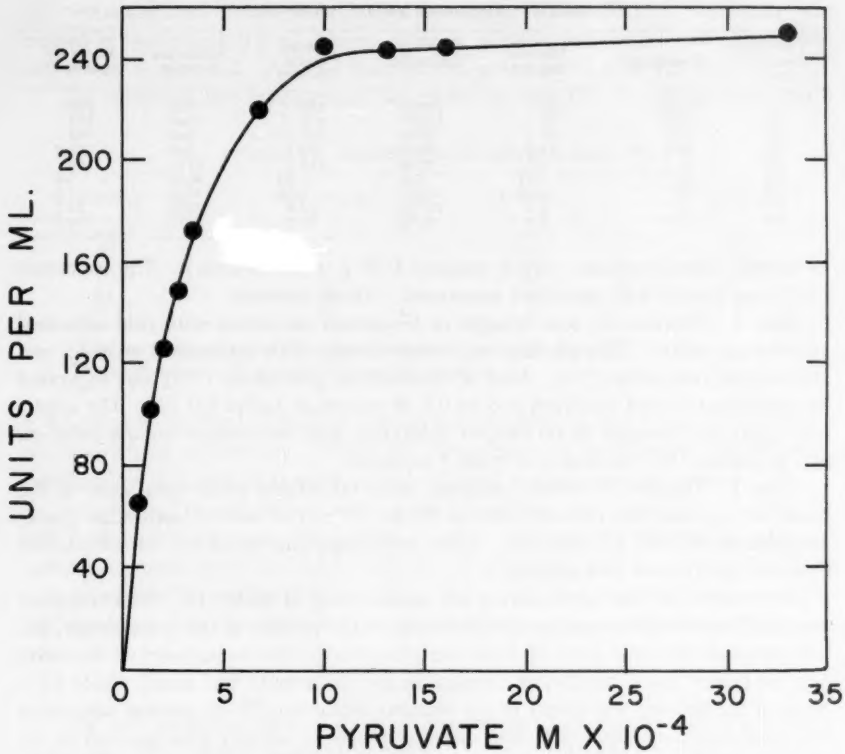

FIGURE 3. Optimum pyruvate concentration. Mixtures contained 0.1 M Tris buffer pH 7.35, 1.0 ml; DPNH solution 2 micromoles per ml, 0.1 ml; 0.01 M pyruvate 0.02 to 1.0 ml; water to make 2.96 ml; reaction started by adding 0.04 ml dilute, centrifuged homogenate containing 0.37 mg protein per ml.

TABLE II. *Inhibition and reversal.*

Additions	Concentration ($M \times 10^{-4}$)	Incubation (minutes)	Inhibition (percent)
none	—	30	0
iodoacetate	10	30	3
iodoacetamide	10	30	0
arsenite	10	30	−9
p-mercuribenzoate	10	30	100
p-mercuribenzoate	10	3	94
p-mercuribenzoate	3.3	3	74
p-mercuribenzoate	3.3	3	—
+ cysteine	33.3		36
p-mercuribenzoate	3.3	3	—
+ BAL	3.3		0

Purification. Ammonium sulfate fractionation. Table III.

Step 1. The homogenate (W) obtained from 3.88 g wet weight of organisms was centrifuged at 27,000g for 5 minutes in a Servall SS1 centrifuge. The head had been cooled to 2° C. The supernate (S1) had a volume of 15 ml and the sediment, which was discarded, weighed 1.35 g. Acetic acid 25 percent was added to S1 to pH 4.5 (0.6 ml) and, after standing for 30 minutes at 2° C, was centrifuged

TABLE III. *Ammonium sulfate fractionation.*

Fraction (designation)	Treatment	Protein (mg/ml)	Units/ml	Total units ($\times 10^3$)	Units/mg protein	Recovery (percent)
W	none	30.7	12600	189	410	100
S1	cent.	12.9	13500	203	1046	107
S2	acid	10.1	8500	127.5	840	68
P3	45% AS	14.0	4810	24.1	343	12.7
P4	66 " "	8.2	2700	113.5	2770	60
P5	29 " "	0.27	820	1.64	3038	0.9
P6	44 " "	0.62	1640	3.28	2645	1.8
P7	55 " "	3.2	10750	43.0	3360	22.7
P8	61 " "	2.3	9375	37.5	4076	20.0

as above. The precipitate, which weighed 0.58 g, was discarded. The supernate (S2) was treated with saturated ammonium sulfate solution.

Step 2. Fraction S2 was brought to 45 percent saturation with cold saturated ammonium sulfate. The addition was made slowly, with mechanical stirring, and temperature control at 2° C. After 30 minutes the precipitate (P3) was separated by centrifugation and dissolved in 5 ml 0.2 M phosphate buffer pH 7.5. The supernate (S3) was brought to 66 percent saturation with ammonium sulfate solution, and precipitate P4 removed and treated as above.

Step 3. Fraction P4 which contained about 60 percent of the total units of the whole homogenate was refractionated at 29, 44, 55, and 61 percent saturation giving precipitates P5, P6, P7, and P8. These were separated as above, dissolved, and analyzed for protein and activity.

The results of this fractionation are summarized in table III. Centrifugation and acid-treatment removed about two-thirds of the protein of the homogenate, but left over half the total units of lactic dehydrogenase. Acid-treatment in this case was not typical since, usually, an increase in specific activity was found (table IV). Most of the activity was found in the fraction containing 55–61 percent saturation with ammonium sulfate. A 10-fold increase in specific activity was obtained in the fraction precipitating in 61 percent saturated ammonium sulfate.

Calcium phosphate gel fractionation. Table IV.

TABLE IV. *Calcium phosphate gel fractionation.*

Fraction	Treatment	Protein (mg/ml)	Units/ml	Total units ($\times 10^3$)	Units/mg protein	Recovery (percent)
W	none	23.2	6870	103	296	100
S1	cent.	9.2	6750	101.3	734	98
S2	acid	3.7	4560	73	1230	71
P3	44% AS	3.9	2550	12.8	654	12
P4	75 " "	5.5	10880	54.4	1980	53
E1	elut.	1.47	3880	7.8	2640	7.5
E2	"	1.33	3690	7.4	2773	7.2
E3	"	0.88	2550	4.5	2556	4.4

Step 1. The homogenate (W) obtained from 2.78 g wet weight of organisms gave a sediment weighing 1.32 g on centrifugation, and one of 0.42 g after acid treatment.

Step 2. Supernate S2 from acid treatment was brought to 44 percent saturation ammonium sulfate, the precipitate (P3) removed and dissolved in 5 ml phosphate buffer. Supernate was brought to 75 percent saturation and precipitate P4 treated as above.

Step 3. Fraction P4 was treated with 0.2 ml 15 percent calcium chloride solution with mechanical stirring at 2° C. After 15 minutes, the gel was removed at

the centrifuge and eluted with 2.0 ml phosphate buffer (E1). Supernate was treated as above with 0.2 ml calcium chloride solution and the second gel removed and eluted to give E2. A third fraction was prepared to give E3.

The results of this fractionation are given in table IV. It can be seen that a

TABLE V. *Stability of lactic dehydrogenase.*

Fraction	Days storage	Percent	Original activity with BAL
S2	0	100	113
S2	7	115	123
S2	14	102	120
F75*	21	103	122
S1	56	85	92
S1**	63	27	41

* Fraction precipitated at 75 percent saturation ammonium sulfate.
** Homogenized in 0.9 percent KCl and centrifuged.

more effective removal of inactive protein was obtained in step 1 than was obtained in the previous experiment. About the same degree of purification was achieved, but the yield was only 30 percent of that obtained with ammonium sulfate.

Stability. The remainders from several fractions were stored in a freezer for different lengths of time. They were thawed and activities determined with and without the addition of BAL 3.3×10^{-3} M. The results in table V show that lactic dehydrogenase is completely stable for several weeks, and that BAL restores about half the loss occurring later. BAL also activates the enzyme slightly in fresh samples.

DISCUSSION

The data presented confirm those of Kupferberg et al (1953) and Wirtschafter and Jahn (1956) that *T. vaginalis* contains a lactic dehydrogenase which is linked to diphosphopyridine nucleotide. In addition a 10-fold purification of the enzyme has been accomplished, and several characteristics have been described.

Purification was not extensive enough to make a profitable study of substrate specificity. However, it was shown that D (−) lactate was not oxidized, whereas the L (+) isomer was readily attacked. Our purest preparations still attacked malate and oxalacetate as well as pyruvate and lactate, but the former activity is associated with malic dehydrogenase. These experiments are now in progress and will be reported later.

Triphosphopyridine nucleotide showed only a fraction of the activity shown by DPN. This finding agrees with those from studies of lactic dehydrogenase from other organisms.

Inhibition studies with sulfhydryl reagents indicated sensitivity toward mercaptide formers (PMB) but not toward alkylating agents (iodoacetate and its amide). This type of differentiation seems to be quite general, most enzymes being more sensitive to mercaptide formers.

Reversal of inhibition by PMB was found with both cysteine and BAL, but the latter was much more effective than the former. It not only gave more rapid reactivation, but more extensive reactivation as well. It even reactivated small amounts of enzyme which had become inactive during preparation or storage of homogenates.

SUMMARY

Lactic dehydrogenase of *Trichomonas vaginalis* is found in supernates of homogenates of the organism and is linked to diphosphopyridine nucleotide. Triphosphopyridine nucleotide gave only 16 percent as much activity as DPN. Only L (+) lactate was oxidized, the D (−) isomer being totally inactive. The optimum pH for pyruvate reduction was 7.6. The concentration of DPNH giving half-maximum activity (K_m) was 1.7×10^{-5} M, and the corresponding concentration of pyruvate was 2×10^{-4} M. The enzyme was fairly stable when stored at 0° C or frozen. Only 15 percent loss of activity was found after 56 days. BAL restored about half of this loss. Activity appears to be related to sulfhydryl groups, since para-mercuribenzoate inhibited completely at 3.3×10^{-4} M. BAL completely reversed this inhibition. Cysteine was less effective. Iodoacetate, iodoacetamide, and arsenite at 1×10^{-3} M concentration had no inhibiting effect even after 30 minutes incubation with the enzyme. About a 10-fold purification of the enzyme was achieved with ammonium sulfate fractionation. The fraction precipitating at 58–66 percent saturation contained 85 percent of the total enzyme. Calcium phosphate gel adsorbed the enzyme, and elution gave a 10-fold purification, but with a smaller yield than ammonium sulfate.

ACKNOWLEDGMENTS

The technical assistance in the preparation of the cultures by Miss Lucy V. Reardon, Mrs. I. L. Bartgis, and Mrs. V. Davis is gratefully acknowledged.

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DIROFILARIA MAGNILARVATUM N. SP. (NEMATODA: FILARIOIDEA) FROM *MACACA IRUS* CUVIER. I. DESCRIPTION OF THE ADULT FILARIAL WORMS

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A microfilaria was described by Poynton and Hodgkin (1939) from a macaque (*Macaca irus*) which was collected in the lower reaches of the Perak river in the state of Perak, Malay. Edeson (1954)* observed microfilariae in the peripheral blood of several *M. irus* collected in the state of Pahang, Malaya, which closely approximated the description given by Poynton and Hodgkin. Adult filarial worms collected later by Dr. Edeson and the author from one of these macaques were determined to belong to the genus *Dirofilaria*.

After returning to the United States, the author had an opportunity to examine at Walter Reed Army Medical Center, Washington, D. C., National Institutes of Health, Bethesda, Maryland, and Eli Lilly Research Laboratories, Indianapolis, Indiana, a total of 510 *M. irus* believed to have originated in Malaya.** In 12 of these monkeys microfilariae were found similar to those previously observed in Malaya. Adult worms were collected also from several of these macaques, and the microfilariae were associated with them in most cases.

A number of species of *Dirofilaria* have been described from the subcutaneous connective tissue of monkeys and apes. Linstow (1899) described *Filaria corynodes* from several species of monkey in Africa. This filaria was later placed in the genus *Dirofilaria* by Railliet and Henry (1911). Sandground (1933) described *D. macacae* from *Macaca assamensis* and *D. pagumae* from *Paguma larvata*. Vuylsteke and Rodhain (1938) described *D. schoutedeni* from *Colobus polykomos*. Webber (1955a, b) described *D. aethiops* from *Cercopithecus aethiops* and *Colobus badius*. Chabaud and Rousselot (1956) placed *D. schoutedeni* and *D. aethiops* in synonymy with *D. corynodes* (Linstow, 1899) and reviewed the *Dirofilaria* of the primates. None of the descriptions of *Dirofilaria* previously known corresponds to the present species recovered from *Macaca irus*. Because of the size of the microfilariae, the name *Dirofilaria magnilarvatum* has been selected for the new species.

Dirofilaria magnilarvatum n. sp.

Measurements of each sex are expressed as an average (4 males, 8 females) followed by corresponding measurements of type.

Description: Dipetalonematidae Wehr, 1935; Dirofilarinae Wehr, 1935; genus *Dirofilaria*

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* Dr. J. F. B. Edeson was at the time Research Officer at the Filariasis Research Laboratory at Kuantan, Pahang, a field laboratory of the Institute for Medical Research, Kuala Lumpur, Federation of Malaya. Through this Institute the initial work on this study was made possible.

** Most of the monkeys were obtained through Trefflich's Bird and Animal Supply and had originated in Singapore from monkeys supplied by dealers listed as Tong, Otemsky, and Siamese Productions.

Railliet and Henry, 1911; subgenus *Nochtiella* Faust, 1937. Bodies of both sexes long and fairly uniform in diameter tapering slightly at extremities. Extremities rounded, tail short, terminating in a small cuticular knob. Phasmids visible in female on either side of knob. Mouth without lips. Head with 8 small but clearly visible cephalic papillae. Amphids lateral and clearly visible. Esophagus not divided into anterior and posterior portions. Cuticle thick with fine, transverse striations. Microfilaria not sheathed and found in blood of host.

Male (holotype): 41.7 (35.0) mm long. Lateral width at nerve ring 0.206 (0.182) mm, at deirids 0.336 (0.324) mm, at junction of esophagus and intestine 0.316 (0.320) mm. Dorsoventral width at nerve ring, 0.186 (0.185) mm, at junction of esophagus and intestine 0.294 (0.322) mm, at anus 0.086 (0.100) mm. Nerve ring 0.234 (0.200) mm from anterior end of body and anus 0.070 (0.055) mm from posterior end. Length of esophagus 1.25 (1.20) mm, of left spicule 0.244 (0.246) mm, of right spicule 0.168 (0.174) mm. Posterior end coiled in 3 to 4 progressively smaller turns. Six pairs of large, lateral, pedunculate, caudal papillae decreasing in size posteriorly, followed by 6 pairs of smaller caudal papillae. Three medial papillae, 1 anterior and 2 posterior to anus.

Female (allotype): 88.0 (90.0) mm long. Lateral width at nerve ring 0.241 (0.255) mm, at deirids 0.422 (0.450) mm, at junction of esophagus and instestine 0.395 (0.470) mm, at vulva 0.413 (0.490) mm. Dorsoventral width at nerve ring 0.240 (0.273) mm, at junction of esophagus and intestine 0.405 (0.470) mm, at vulva 0.425 (0.495) mm, at anus 0.117 (0.119) mm. Vulva in the region of the junction of the esophagus and intestine 1.75 (2.04) mm from the anterior end. Nerve ring 0.281 (0.314) mm from anterior end and anus 0.066 (0.056) mm from posterior end. Esophagus 1.52 (1.83) mm long. Opisthodelphys; viviparous.

Host: Macaca irus Cuvier.

Location: Subcutaneous connective tissue.

Type Locality: Cherok Paloh, Pahang, Malaya.

Holotype and Allotype: U. S. N. M. Helm. Coll. No. *37266*.

Paratypes: (2 males and 4 females) U. S. N. M. Helm. Coll. No. *37267*.

Distribution: States of Perak and Pahang, Federation of Malaya (probably the state of Johore also from where it is believed the collectors obtained most of their monkeys).

Diagnosis: A *Dirofilaria* with microfilaria about 580 microns long (Taylor, 1959). Posterior end of male with 12 pairs of caudal papillae and 3 medial papillae. Distal tip of left spicule split and covered by a fine membrane. Female with large extraesophageal glands. This combination of characters is diagnostic for *Dirofilaria magnilarvatum*.

DISCUSSION

The esophagus of the female has no apparent glandular region. However, running parallel to it and almost surrounding the esophagus, there is a distinct glandular area in the region of the nerve ring. The function of this glandular structure is not known. No direct connection to the exterior or to the esophagus was observed. Since this is a tissue-migrating species, the glands may have a cytolytic action, thereby enabling the worm to move through the tissue more freely.

In the present study it has been necessary to measure each adult filarial worm to confirm the opinion that the worms in question are a new species and separate from a second species which was found in some of the macaques examined. The range of length for the 8 females examined was from 79.0 mm to 101.0 mm; and for the 4 males, 33.0 mm to 51.0 mm, the males being nearly half the length of the females. Females of the described species were found deep in the subcutaneous tissue and did not come off with the skin when it was removed.

Some adult females were found in the subcutaneous connective tissue close to the surface. In one case the female was visible through the intact skin (pl. II). Subsequently 3 adult female worms 175, 190, and 214 millimeters long, respectively, were recovered. Although, by morphological features other than size, these could not be separated from the new species described, the author feles after a study of all the female worms that these are representative of another species of *Dirofilaria*. The fact that 2 different unsheathed microfilariae were observed, one averaging 375 microns and the other averaging 580 microns in length, establishes that 2 species of

filaria were present. Since no male worms were recovered, the author has decided not to describe the second species, but only to point out its existence in *M. irus* in the hope that males as well as females and microfilariae may be found.

Since animals are usually determined to be infected by finding microfilariae in the blood or body fluids, it is unfortunate that it is not considered essential to include a description of the microfilariae along with that of the adult worms. The importance of microfilarial descriptions and the establishment of standard techniques for determining the species present in animals is suggested by the work of Newton and Wright (1956). Further studies will probably reveal that the records of fleas acting as vectors for *Dirofilaria immitis* are in error, and that at least one species of *Dipetalonema* in dogs uses the flea as an intermediate host. In the present study the measurements of the microfilariae found by the author and by Taylor are relatively close to those given by Poynton and Hodgkin (1939). The measurements should not be expected to correspond exactly, since there is some variation in the results of any 2 individuals and since different procedures were used to prepare the microfilariae for examination. It is certainly better to use morphological characters along with measurements to arrive at a definite conclusion as to the species involved. As Sandground (1933) pointed out, the structure of the genus *Dirofilaria* is extremely simple, and very few characters other than size are consistent enough to be used in species determination. It is possible that a study of the microfilariae of various species might lead to a more suitable method of separating species at least when used in conjunction with adult characters.

SUMMARY

Dirofilaria magnilarvatum n. sp. infecting the subcutaneous connective tissue of *Macaca irus* Cuvier is described from specimens recovered from a monkey in Pahang, Malaya, and monkeys examined in the United States which were probably collected in Malaya. The new species has an extremely large microfilaria averaging about 580 microns in length.

Adult female worms and microfilariae of another species of *Dirofilaria* were recovered also from the subcutaneous connective tissue of 2 monkeys, but were not described.

The importance of complete descriptions of the microfilariae and the establishment of standard techniques in the study of the filaria is emphasized.

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PLATE I

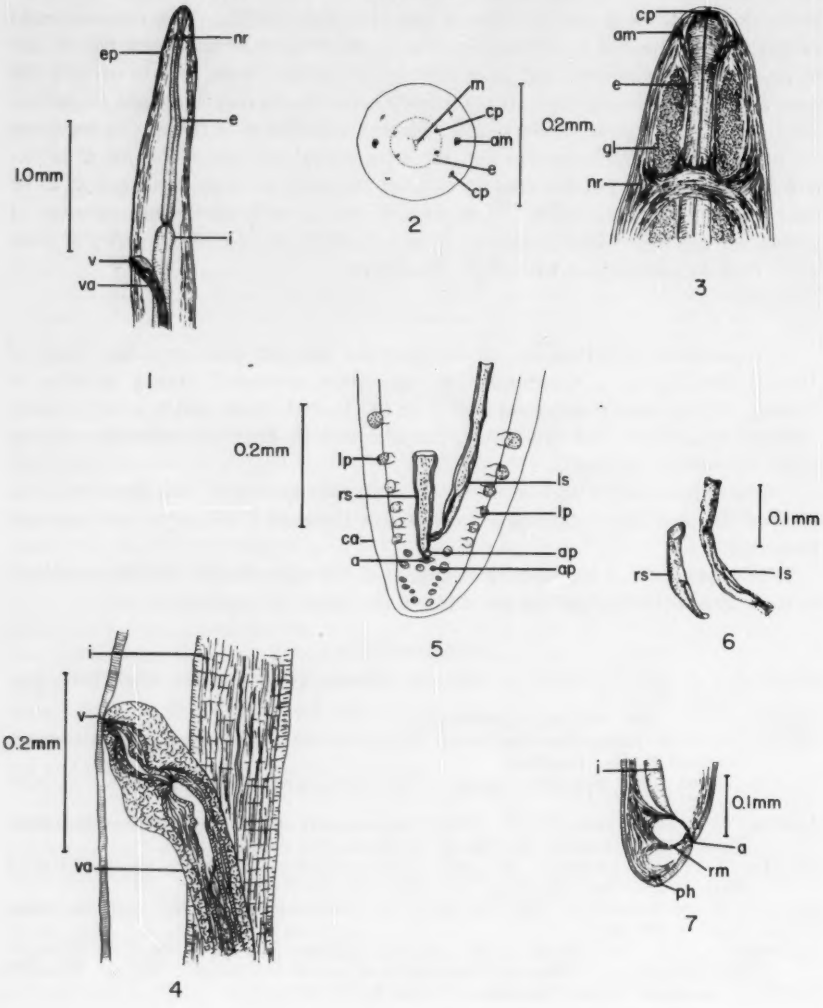

PLATE II

Explanation of Plates

Plate I

Dirofilaria magnilarvatum n. sp. Figures drawn with the aid of a microviewscope.

Figure 1. Lateral view of anterior end of female.
Figure 2. *En face* view of female.
Figure 3. Dorsal view of anterior extremity of female.
Figure 4. Lateral view of vulva and vagina of female.
Figure 5. Ventral view of posterior end of male.
Figure 6. Off-right lateral view of spicules.
Figure 7. Right lateral view of posterior end of female.

Abbreviations: a, anus; am, amphid; ap, anal papilla; ca, caudal alae; cp, cephalic papilla; e, esophagus; ep, excretory pore; gl, glandular area; i, intestine; lp, lateral papilla; ls, left spicule; m, mouth; nr, nerve ring; o, ovary; ph, phasmid; rm, rectal muscle; rs, right spicule; t, testes; u, uterus; v, vulva; va, vagina.

Plate II

Adult female of undescribed species visible through intact-skin (see arrow) in the inguinal region of male *Macaca irus*.

(Photograph by Eli Lilly Company, Photographic Department.)

RESEARCH NOTE

ORYZAEPHILUS SURINAMENSIS (L.): A NEW INTERMEDIATE HOST FOR THE CESTODE *HYMENOLEPIS DIMINUTA*.

Sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.), were brought into this laboratory by a friend who had found them in a package of mixture used in the preparation of fried chicken. Cultures of this beetle were maintained in the laboratory at room temperature in the original mixture, to which flour was later added. Although many different arthropods are known to serve as intermediate hosts for *Hymenolepis diminuta*, no reference to the sawtoothed grain beetle as an intermediate host has been found.

Infection experiments were carried out as previously described for *Tribolium confusum* (Voge, M. and Heyneman, D., 1957, Univ. Calif. Publ. Zool. **59**: 549–580). Eight days after exposure to the tapeworm eggs, beetles were found to contain cysticercoids which were infective to rats. In appearance, cysticercoids from *Oryzaephilus* differed from those developed in *T. confusum* by being smaller in overall size and somewhat distorted. At 9 or 10 days of development, no change in appearance was observed. Repeated experiments produced similar results. Attempts to infect these beetles with *Hymenolepis nana* were unsuccessful except for the recovery of one abnormal cysticercoid.

While *O. surinamensis* is a suitable laboratory host for *H. diminuta* in that infections are not difficult to produce and cultivation of beetles is easily accomplished, problems arise in the handling of these organisms. *Oryzaephilus* is much smaller and faster than *Tribolium*, and can escape from almost any covered glass dish which is not tightly sealed with paraffin or scotch tape. Moreover, this beetle can easily move along any vertical or inverted glass surface.

Concurrently with the above experiments, we attempted to infect the drug store beetle, *Segobium panaceum* (L.), with *H. diminuta* and *H. nana*. This beetle was recovered from tropical fish food, and a culture established in flour and pulverized Purina rat chow. The drug store beetle is apparently quite sensitive to changes in environment and does not survive temperatures at 30° C or higher. All attempts to infect this beetle were unsuccessful.

Thanks are extended to Dr. John Belkin of this University for identifying the beetles.—
Mary T. Mullee and Marietta Voge, *Department of Infectious Diseases, School of Medicine, University of California, Los Angeles.*

DIROFILARIA MAGNILARVATUM PRICE, 1959 (NEMATODA: FILARIOIDEA) FROM *MACACA IRUS* CUVIER. II. MICROSCOPICAL STUDIES ON THE MICROFILARIAE

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National Institute for Medical Research, Mill Hill, London, N. W. 7

This paper describes the microfilaria of *Dirofilaria magnilarvatum* and compares it with that of *Dirofilaria immitis*.

METHODS

Infected tail blood was obtained, by the method described by Hawking (1959), from 2 Malayan monkeys (*Macaca irus*) kept at this Institute. The microfilariae were separated from blood cells, photographed alive, and stained by a technique described previously (Taylor, 1959). Briefly, this was as follows: A small drop of heparinized infected blood was placed in a pool of homologous serum on a slide and left covered for about 10 minutes. After the blood cells had settled down on the slide, the microfilariae wriggled free into the clear serum where they could be picked up with a micropipette. These embryos were mounted on an agar pad on a slide and a coverslip was gently lowered onto the preparation. The agar pad acts as a cushion which pre-

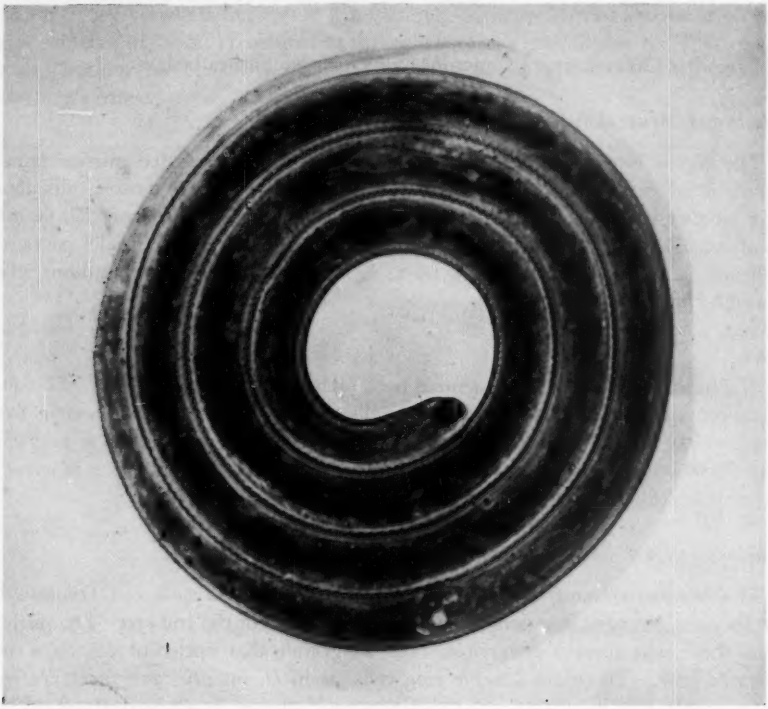

FIGURE 1. Microfilaria of *D. magnilarvatum* photographed alive in ultra-violet. ×1500.

Received for publication January 12, 1959.

vents the specimens from bursting under the pressure of the coverslip. The microfilariae thus freed from the obscuring presence of blood cells were ready for examination with the phase-contrast microscope.

Some microfilariae were photographed alive (fig. 1) using the ultraviolet microscope (2750 A), the essential difference in technique being the use of quartz slides and coverslips. A few microfilariae were fixed with Bouin's fluid and stained with Giemsa for comparison with the living specimens (fig. 3).

RESULTS

These microfilariae live freely in the blood of the host, and do not possess a sheath (fig. 2). They are much larger (580 ± 10 microns by 8 microns) than those

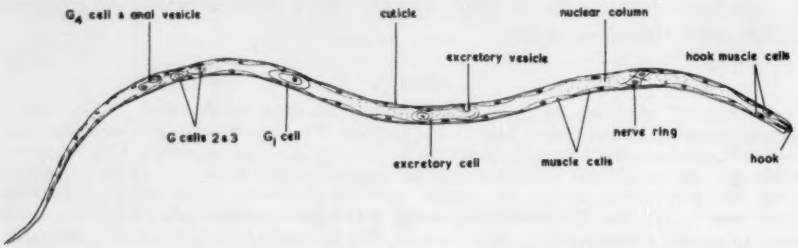

FIGURE 2. Microfilaria of *D. magnilarvatum*, schematic drawing showing anatomical structures.

of *D. immitis* (260 microns $\pm$ 5 microns) which is also unsheathed.

Cuticle and "Musculature".

The cuticle is striated, and the width between striations is 0.8 micron (in *D. immitis* it is 0.6 micron). Underlying the cuticle are very large muscle cells about 16 in number lying along the whole length of each side of the embryo. *D. immitis* has about 23 muscle cells along each side. The muscle cell nuclei usually contain 1 nucleolus each. These nuclei show up as light areas in the dark cytoplasm when viewed by phase-contrast.

Head.

The head is blunt, and bears a small hook attached to a cuticular disc. This disc is connected by fibrils to 2 large cephalic cells. These cells, as in *D. immitis*, have elongated nuclei, and their cytoplasm extends forward towards the hook and contains fibrils which were seen, by phase-contrast, to contract during the movement of the hook. These cells are poorly absorbing in ultra-violet light.

"Nuclear-column" and Nerve Ring.

The "nuclear-column" cells are similar in arrangement to those of *D. immitis*, in that they are arranged in a double row along the length of the embryo. The nuclear sap of these cells absorbs ultra-violet rays, suggesting that nucleo-proteins are concentrated here. There are 2 nerve ring cells, as in *D. immitis*, and these are best seen when they are lightly stained with Giemsa and viewed by phase-contrast. They are diamond-shaped and have large nuclei usually containing 1 nucleolus. The nerve ring lies further from the head than does that of *D. immitis* (see table I).

Excretory Apparatus.

The excretory cell is the largest cell of the body in both *D. immitis* and the microfilaria of *D. magnilarvatum*, although the nucleus of this cell is not as large as that of the G_1 cell in either organism. The excretory cells of both species are very

TABLE I. *Dimensions of the microfilaria of* D. magnilarvatum, D. immitis *and the microfilaria described by Poynton and Hodgkin (1939). (Measurements in microns.)*

	D. magnilarvatum	*D. immitis*	Poynton & Hodgkin's microfilaria
Length	580 ± 10	260 ± 5	600
Width	8	4.5	5
Width of cuticular striations	0.8	0.6	
Number of striations per micron	1.2	1.7	
	Percent distance from anterior end		
Nerve ring	23.5	19.2	17.6
Excretory pore	27.5	29.75	24.25
Excretory cell	32.0	39.5	
G_1 cell	77.0	62.0	
G_4 cell	89.0	73.0	
Anal pore	86.5	75.0	77.0

long (about 30 microns) and their nuclei usually contain 1 basophilic nucleolus. This cell lies nearer the head in the microfilaria of *D. magnilarvatum* than it does in *D. immitis*. The cytoplasm of the excretory cell has a fluid movement during life, and it envelops the excretory vesicle anteriorly (about 160 microns from the head). The excretory vesicle is bulbous in both species. Phase-contrast examination provides evidence of "villi" structures in the vesicle of the microfilaria of *D. magnilarvatum* (but not that of *D. immitis*), their purpose presumably being to increase the excretory surface.

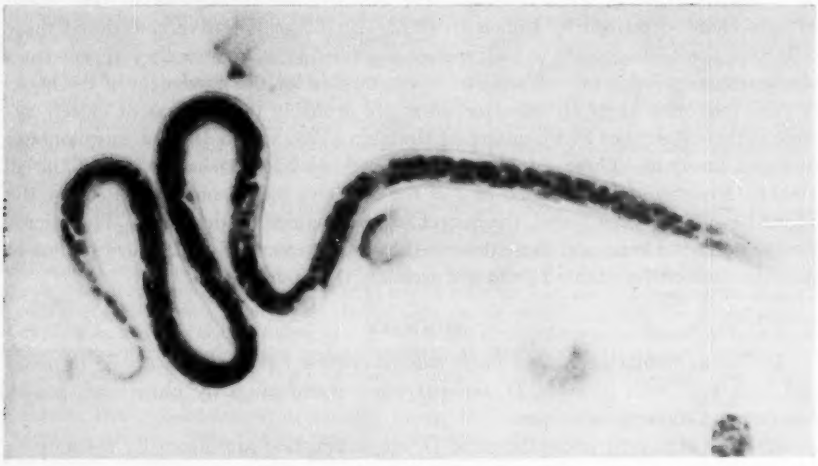

FIGURE 3. Microfilaria of *D. immitis* fixed with Bouin and stained with Giemsa. ×1100.

G cells and Anal Pore.

The nucleus of the G_1 cell of both species is very large and that of the microfilaria of *D. magnilarvatum* frequently contains 2 nucleoli. The relative positions of the G cells and the anal pore differ in the 2 species (see table I). The position of

the G_4 cell of the microfilaria of *D. magnilarvatum* is unusual because it lies posterior to the anal pore, whereas in *D. immitis* and most other microfilariae it lies anterior to the anal pore. In both species the cytoplasm of the G_4 cell envelopes the anal vesicle which, in the Malayan microfilaria only, contains 2 highly refractile bodies. The function of these bodies is unknown. The anal vesicle is smaller than the excretory vesicle in both species and does not contain "villi" structures.

The tail of the microfilaria of *D. magnilarvatum* is longer and more tapering than that of *D. immitis*, but in both species the cells of the "nuclear-column" extend almost to the tip of the tail.

DISCUSSION

Microfilariae of *D. magnilarvatum* are unusual in several ways. They are relatively large organisms whose size seems to be due to the greater size of their individual cells, especially the muscle cells, rather than to an increased number of cells in each microfilaria. Another unusual feature is their resistance to ultra-violet rays. These microfilariae can withstand exposures of up to 5 seconds (from the ultra-violet source in the microscope) and still remain motile (two seconds' exposure is generally sufficient to immobilise *D. immitis* and *Loa loa*). Ultra-violet photomicrographs of them show little contrast, possibly because they are too large for the penetration of ultra-violet rays or perhaps because they have rather poorly absorbing nuclei. One further difference between these microfilariae and those of other species lies in the position of the G_4 cell. In the microfilariae of *D. magnilarvatum* the G_4 cell lies posterior to the anal vesicle, whereas in *D. immitis, L. loa, D. repens, Wuchereria bancrofti, W. malayi* and *D. aethiops* it lies close, but anterior to the anal vesicle.

The contractile cephalic cells of these microfilariae correspond to the "roten Mundgebilde" described by Fülleborn (1929) in *L. loa.* I have considered these cells to be hook-muscle cells in both species described here, because they appear from observations on living microfilariae to be responsible for the movement of the hook.

The microfilariae of *D. magnilarvatum* are probably the same as or closely related to those described by Poynton and Hodgkin (1939) from *Macaca irus* monkeys of Kuala Lumpur. These workers found some very large unsheathed microfilariae (600 by 5 microns) in the blood of 2 of the monkeys they examined. Because the adults have never been found, the microfilariae remained unidentified. The microfilariae described here, and those described by Poynton and Hodgkin, are similar in their dimensions (see table I) and are probably the same species.

SUMMARY

1. The microfilariae of *Dirofilaria magnilarvatum* have been described in detail and compared with those of *D. immitis* after examination by ultraviolet, phase-contrast and staining techniques.

2. The [Malayan] microfilariae of *D. magnilarvatum* are unusually resistant to ultra-violet irradiation. They are relatively large (580 ± 10 microns long) and their cells are larger than those of most microfilariae.

3. The "roten Mundgebilde" have been renamed hook-muscle cells because of their proximity to the hook and the part they play in the movement of it.

4. Two nerve cells have been differentiated by light staining with Giemsa, and they can be seen in ultra-violet photomicrographs.

ACKNOWLEDGMENTS

My thanks are due to Dr. F. Hawking in whose laboratory this work was carried out, to Mr. J. Smiles for advice and encouragement and to Mr. J. Clarke and Mr. F. Wanless for technical assistance. The author is also grateful to Dr. J. F. B. Edeson for supplying the monkeys.

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RESEARCH NOTE

AUCHENIA GLAMA AND *ANTILOPE CERVICAPRA*, NEW HOSTS FOR SOME TRICHOSTRONGYLIDAE.

Trichostrongylids collected from the fourth stomach of a llama (*Auchenia glama*) which died in a zoological garden in the Netherlands were identified as *Haemonchus contortus* (Rudolphi, 1803), *Trichostrongylus axei* (Cobbold, 1879), *Ostertagia leptospicularis* Asadov, 1953, *Ostertagia mossi* Dikmans, 1931, *Ostertagia circumcincta* (Stadelmann, 1894), *Ostertagia trifurcata* Ransom, 1907, *Teladorsagia davtiani* Andreeva and Satubaldin, 1954, *Grosspiculagia lasensis* (Asadov, 1953), *Camelostrongylus mentulatus* (Railliet and Henry, 1909), *Spiculopteragia spiculoptera* (Gushanskaia, 1931), *Spiculopteragia asymmetrica* (Ware, 1925) and *Apteragia quadrispiculata* Jansen, 1958. So far as the writer is aware these trichostrongylids have not been reported from the llama before, though Arnao Mendoza (1951, Rev. Inst. Nac. Biol. Animal **2:** 76–81) reported *Trichostrongylus* sp. from the intestine of a llama, and Teuscher and Stunzi (1956, Acta Trop. **13:** 262–269) identified eggs of *Haemonchus* sp. and *Trichostrongylus* sp. in llama feces.

A. quadrispiculata is a little known trichostrongylid. It belongs to the genus *Apteragia* Jansen, 1958, of the subfamily Trichostrongylinae Leiper, 1908. The males of *A. quadrispiculata* measure 3.8–7.0 mm, their maximum width being 0.081–0.121 mm. Length of oesophagus 0.380–0.586 mm. Cervical papillae very small. Length of left spicule 0.186–0.226 mm, length of right spicule 0.182–0.222 mm. The spicules divide into 4 branches. In the left spicule, the ventral branch ends with a medially curved tip; in the right spicule, with 2 proximally recurved points, resembling an anchor. Gubernaculum absent. (For full description see Jansen, 1958, Lebmaagtrichostrongyliden bij Nederlandse herten. Proefschrift, Rijks-Univ., Utrecht.)

Trichostrongylids collected from the fourth stomach of an antelope (*Antilope cervicapra*) which died in a zoological garden in the Netherlands, were identified as *Trichostrongylus axei* (Cobbold, 1879), *Trichostrongylus vitrinus* Looss, 1905, and *Camelostrongylus mentulatus* (Railliet and Henry, 1909). In one male of *C. mentulatus* the spicules were missing, probably because of an accident.

Trichostrongylids collected from the small intestine were identified as *Trichostrongylus colubriformis* (Giles, 1892), *Trichostrongylus probolurus* (Railliet, 1896), *Trichostrongylus vitrinus* Looss, 1905, and *Nematodirus spathiger* (Railliet, 1896).

Sloan (1951, Proc. Zool. Soc. London **121:** 723–725) reported *H. contortus, O. circumcincta, O. trifurcata, T. retortaeformis, T. axei, T. colubriformis* and *T. vitrinus* from *A. cervicapra*. So far as the writer is aware, *T. probolurus, C. mentulatus* and *N. spathiger* have not been reported from *Antilope cervicapra* before.—JACOB JANSEN JR., *Institute of Veterinary Parasitology and Parasitic Diseases of the State University, Utrecht, the Netherlands.*

RESEARCH NOTES

REPORT OF A POSSIBLE HUMAN INFECTION WITH THE ACANTHOCEPHALAN *MONILIFORMIS MONILIFORMIS* (SYN. *M. DUBIUS*)

A mature male acanthocephalan, *Moniliformis moniliformis* (syn. *M. dubius*) measuring approximately 7 cm in length was sent to this laboratory by the attending physician for identification. This parasite presumably had been vomited by a patient during a period of illness. Direct questioning of the patient by the author revealed that during the night she had a "gallbladder attack" with accompanying nausea and vomiting. Several clean towels were placed near the patient's mouth to collect the vomitus and thus protect the bed clothing. The following morning the towels, with the vomitus wrapped within, were placed in the automatic washing machine and washed. Large amounts of mucus with enmeshed undigested food apparently prevented proper drainage, so the contents remaining were removed by hand. Observation by the attending married daughter revealed the above-mentioned parasite in the debris. It was saved and given to the attending physician.

Subsequent repeated stool examinations of the patient in question and her daughter, who cared for her, failed to reveal ova, larvae or adult worms of any kind in either person.

The circumstances surrounding the discovery of this parasite might raise doubts as to the authenticity of the infection, but the case, in the opinion of the author, warrants reporting.

A difference of opinion exists as to the proper classification of the genus *Moniliformis*, parasitic in rats in the U. S. While Van Cleave (1953, Ill. Biol. Monog. 23) considers *M. moniliformis* the proper species name with *M. dubius* a synonym, Chandler (1955) in his text adheres to *M. dubius* as a distinct species. Reports of experimental work on the species in the United States mention only cockroaches as the intermediate host, while European reports refer to beetles as the intermediate hosts there.

Since cockroaches and coprophagous beetles have an extensive distribution in tropical climes along with wild rodents, the rare occurrence of a human infection can thus be accounted for. It is highly improbable that any rodent could have had free access to the washing machine, not to mention the elimination of the parasite during such a visit. The ingestion of an infected intermediate host in food is possible. Since Acanthocephala (Chandler, 1955) have a propensity for reestablishing themselves as larvae in abnormal hosts, human infections might conceivably occur without the necessity of eating roaches or beetles. The significance of this report lies in the rarity of such infections. Only 3 naturally occurring infections have been previously reported in man: One from Italy, one from the Sudan, and one from British Honduras.—J. WALTER BECK, *Department of Microbiology, University of Miami, School of Medicine, Coral Gables, Florida.*

SPIROCERCA LUPI FROM A NEW HOST, THE JAGUAR.

Spirocerca lupi, a common nematode parasite of dogs in many areas of the world, has been reported from the wolf, jackal, and fox, and also from the cat in experimental infections. The purpose of this note is to report a case of natural infection of *S. lupi* in the jaguar, *Felis onca*, the first such infection reported in the literature so far as could be determined by an examination of the records of the Index of Medical and Veterinary Zoology (Animal Disease and Parasite Research Branch, U. S. Dept. of Agriculture, Beltsville, Maryland).

The jaguar, a female reportedly 3 months old, was purchased October 2, 1958, from a pet farm which had imported it from South America. She died suddenly 4 days later without having shown signs of illness until the brief struggle which preceded death.

On autopsy examination, the lesions were found to be confined to the thoracic cavity. They consisted of: multiple aneurysms of the thoracic aorta (characteristic of those produced by migrating *Spirocerca* larvae), perforation of the aorta in one of the affected areas with resulting hemothorax, and the presence in the clotted blood of the aorta or in the wall itself of at least 6 immature nematodes three-fourth to 1 inch in length. These were identified as *Spirocerca lupi* and placed in the U. S. National Museum Helminthology Collection (No. 37263).

Occasional cases of spontaneous rupture of the aorta as seen in this case have been reported from dogs with *Spirocerca* infection. We have seen no more than 3 such cases during a period of 7 years during which time nearly 2000 dogs (6 months or older) were autopsied, approximately 8 percent of which had *Spirocerca* infection.

Definitive information is lacking on the date this animal was shipped from South America as well as on the time required for migration and development of *Spirocerca* larvae, but the available information would indicate that this infection was acquired in South America.—W. S. BAILEY, *Department of Pathology and Parasitology, School of Veterinary Medicine, Alabama Polytechnic Institute.*

DIROFILARIA MAGNILARVATUM PRICE, 1959 (NEMATODA: FILARIOIDEA) FROM *MACACA IRUS* CUVIER. III. THE BEHAVIOR OF THE MICROFILARIAE IN THE MAMMALIAN HOST

FRANK HAWKING

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This paper reports observations on the behavior and distribution of the microfilariae of *Dirofilaria magnilarvatum*. These microfilariae are remarkable for their great length, 580 microns. Also, their behavior in the body of the host differs from that of other microfilariae which we have studied.

On July 9, 1956 two small *Macaca irus* monkeys (Nos. 285 and 286) were received from Malaya through the kindness of Dr. J. F. B. Edeson. The blood contained malaria parasites and microfilariae of the species in question. The blood was examined at weekly intervals. The number of microfilariae was irregular, but showed no consistent changes over many months. No evidence of diurnal periodicity could be detected.

The monkeys were exposed to various stimuli which we had previously found to have a marked effect in increasing (and decreasing) the count of other microfilariae in the peripheral blood (Hawking 1956). These included anaesthetization with ether or nembutal, breathing oxygen, breathing 10 percent oxygen in nitrogen (anoxia), breathing high carbon dioxide atmospheres, and exercise; but there was no appreciable change in the number of microfilariae obtained from the ear or tail. In monkey 285, the number of microfilariae in 20 cu mm of blood taken from the ear ranged from 0 to 11. Often there was great contrast between the first and second samples collected from the same puncture. If, however, the tail was allowed to hang down, and the end became cold and the blood presumably stagnant, samples of blood collected from the end of it contained up to 165 microfilariae per 20 cu mm. In the case of monkey 286, samples from the ear contained 1 to 4 microfilariae per 20 cu mm, but samples collected from the end of the dependent tail contained up to 190 microfilariae per 20 cu mm.

During an experiment (October 3, 1956) in which monkey 285 had been given ether and exposed to oxygen, a small piece of skin was excised from the dorsum of the foot. At this time blood from the ear contained only one microfilaria in 80 cu mm. The skin was fixed in formalin and examined in histological section. Seven segments of microfilariae were found in 31 small vessels which seemed to be arterioles, but none in 41 venules or 33 capillaries. In a later experiment (October 24, 1956), the same monkey was examined under anaesthetic and small pieces of tissue were taken. Blood from the ear contained 6 microfilariae in 80 cu mm; blood from the end of the tail, 288 microfilariae in 80 cu mm; blood from a leg vein, 1 microfilaria in 80 cu mm. Skin from the epigastrium and from the thigh showed no microfilariae in histological section. Tissue from the thigh contained no microfilariae in skeletal muscle (25 sq mm) or adipose tissue (17 sq mm). The skin from the end of the tail contained 4 sections of microfilariae in 34 arterioles,

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but none in 78 veins or 45 capillaries. The microfilariae were lying loosely in the lumina of the vessels, and there was room for them to curl and turn back. These observations suggest that the microfilariae (possibly on account of their great length) tend to congregate in the arterioles of some parts of the body, especially the skin of dependent parts.

The monkeys continued to be kept in the laboratory with periodic examinations, and there seemed no obvious change in their microfilarial counts up to July 9, 1957, when monkey 286 showed 8 microfilariae in 40 cu mm of blood from the ear and 240 microfilariae in 40 cu mm from the tail. However, by September 3, 1958, this monkey showed 5 microfilariae in 40 cu mm of blood from the ear and only 10 microfilariae in 40 cu mm of blood from the tail. On September 19, 1957, the spleen was excised from monkey 285 (to see if this influenced the microfilaria count). Smears from the spleen showed no microfilariae and blood taken from the ear on four occasions (September 27 to October 7) showed no microfilariae in 160 cu mm. On October 15 the monkey had a severe infection of *Plasmodium knowlesi* (presumably due to the splenectomy) and died in spite of treatment. Smears made from the lungs and other organs showed no microfilariae and careful search disclosed no adult worms.

ACKNOWLEDGMENTS

Grateful acknowledgments are due to Dr. J. F. B. Edeson for the supply of the monkeys and to Mr. K. Gammage for technical assistance.

SUMMARY

A study was made of the behavior in the host of unusually long microfilariae (*Dirofilaria magnilarvatum*) found in *Macaca* irus monkeys. The microfilariae showed no diurnal periodicity; they did not respond to stimuli such as anaesthetic or changes of oxygen pressure which affect the distribution of other microfilariae; they tended to accumulate in the skin at the end of the tail, especially in arterioles.

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DIROFILARIA MAGNILARVATUM PRICE, 1959 (NEMATODA: FILARIOIDEA) FROM *MACACA IRUS* CUVIER. IV. NOTES ON LARVAL DEVELOPMENT IN MANSONIOIDES MOSQUITOES

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Two species of microfilariae were reported in the blood of the long-tailed macaque, *Macaca irus,* by Poynton and Hodgkin (1939). One of these microfilariae was very long (about 600 microns) and unsheathed, while the other was shorter (about 200 microns), sheathed, and indistinguishable from the microfilariae of *Wuchereria malayi.* Microfilariae reported from *M. irus* by Edeson, Wharton and Buckley (1955) and Price and Jachowski (1955) were presumably the same species as those described by Poynton and Hodgkin. Subsequently, adult worms associated with the large microfilariae were recovered from subcutaneous connective tissue and fascial planes between muscles and assigned to the genus *Dirofilaria* by Price (1959) under the name *D. magnilarvatum.* The present notes report observations of the development of the microfilariae in the mosquitoes, *Mansonia* (*Mansonioides*) *longipalpis* and *M. uniformis.*

The name *M. longipalpis* refers to two very similar forms, *longipalpis* and *bonneae,* which occur together and in which the development of *W. malayi* has been found to be identical (Wharton, 1957). The taxonomic position of the two forms is under investigation and the reasons for retaining the name *M. longipalpis* at this stage are discussed by Wharton (1957a).

MATERIALS AND METHODS

The monkeys were caught in swamp-forest areas in East Pahang, Malaya, where filariasis due to *W. malayi* is highly endemic in the human population. Most studies on the development of *D. magnilarvatum* in mosquitoes were made using macaques infected with both this species and *W. malayi.* Methods were similar to those described by Wharton (1957, 1957a) concerning the development of *W. malayi* in *M. longipalpis.* A 60 cu mm blood sample taken before and after a feeding experiment was used as an estimate of microfilarial density (expressed as the average number per cu mm.). Monkeys were anaesthetized with intraperitoneal nembutal for exposure to wild-caught *M. longipalpis,* and laboratory-bred *M. uniformis.* Larvae from mosquitoes were killed in Bles' fluid and mounted on ringed slides in an aqueous solution containing 10 percent formalin, 5 percent glycerine, and 0.001 percent methylene blue.

OBSERVATIONS

Intake of Microfilariae

Results made on intake of microfilariae of *D. magnilarvatum* by *M. longipalpis* were quite different from those made on *W. malayi* in the same mosquitoes. Wharton (1957) found that intake of *W. malayi* was related to microfilarial density, and that *M. longipalpis* ingested more microfilariae than would have been expected from the size of the blood meal. On the average the number of microfilariae ingested was equivalent to the number present in 5 cu mm of peripheral blood. The results of two experiments in which *M. longipalpis* and *M. uniformis* fed on a monkey with both *W. malayi* and *D. magnilarvatum* are summarized in table I. Of 12 *M. longi-*

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TABLE I. *Numbers of* D. magnilarvatum *and* W. malayi *microfilariae found in the stomach of* M. longipalpis *and* M. uniformis *mosquitoes immediately after feeding on a macaque,* Macaca irus, *infected with both parasites.*

Species of filaria	No. of microfilariae in donor		No. in individual mosquitoes												No. mosq. with mf.	Mean no. per mosq.
	per 60 cu mm	per cu mm	1	2	3	4	5	6	7	8	9	10	11	12		
M. longipalpis																
D. magnilarvatum	599–195	6.6	0	0	5	0	0	0	6	0	0	8	32	0	4/12	4.3
W. malayi	37–121	1.3	0	1	2	4	6	8	8	10	11	13	14	16	11/12	7.8
M. uniformis																
D. magnilarvatum	12–10	0.2	0	7	6	5	0	0	6	1	15	230	—	—	7/10	27.0
W. malayi	62–50	0.9	0	1	2	3	4	5	7	13	16	18	—	—	9/10	6.9

palpis which fed on a monkey with 1.3 *W. malayi* per cu mm, 11 ingested microfilariae. The mean number of microfilariae taken up was 7.8, which was equivalent to the number in 6 cu mm of peripheral blood. The microfilarial counts of *D. magnilarvatum* in the blood were much higher than those of *W. malayi,* but only 4 out of 12 mosquitoes ingested microfilariae. The intake of *W. malayi* microfilariae by *M. uniformis* was comparable to that by *M. longipalpis.* In the case of *D. magnilarvatum* infection with very few microfilariae present in the blood, 7 out of 10 *M. uniformis* ingested microfilariae, one containing approximately 230. From these observations it would appear that in the case of *D. magnilarvatum* the microfilarial count made at the time of feeding had no relation to the number of mosquitoes that would ingest microfilariae or the number of microfilariae ingested by each mosquito. Edeson (pers. comm.) and Hawking (1959) found that the number of microfllariae present in blood samples taken from various parts of the body at the same time varied enormously. Therefore, the microfilarial counts do not reflect the number of microfilariae present in the blood at the biting site, but only those present in the area from which the sample was taken. Such a condition makes the assessment of mosquito susceptibility to infection a difficult task.

Larval Development in Mosquitoes

Most observations of larval development were made on mosquitoes which fed on the monkey with both *D. magnilarvatum* and *W. malayi.* The results are summarized in table II. The larvae of *D. magnilarvatum* develop exclusively in the

TABLE II. *Numbers of* D. magnilarvatum *and* W. malayi *larvae found in* M. longipalpis *and* M. uniformis *mosquitoes fed on an infected macaque,* Macaca irus, *7.5 to 13.5 days previously.*

Days after feed	Number dissected	Infected with *D. magnilarvatum*				Infected with *W. malayi*			
		No.	No. of larvae			No.	No. of larvae		
			st. I	st. II	st. III		st. I	st. II	st. III
M. longipalpis									
8.5	5	4	28	6	–	4	19	9	–
10.5	10	1	–	1	2	8	4	10	37
12.5	43	9	–	1	47	35	11	26	275
TOTAL	58	14 (24 percent)				47 (81 percent)			
M. uniformis									
7.5– 8.5	4	1	–	1	–	4	–	31	4
9.5–10.5	61	13	20	57	–	54	7	23	352
12.5–13.5	7	1	–	–	2	7	1	1	18
TOTAL	72	15 (21 percent)				65 (90 percent)			

Malpighian tubules, as do also the larvae of *D. immitis.*

M. longipalpis is apparently a fairly efficient intermediate host for *D. magnilarvatum*. Examination of stomach contents showed that about 33 percent had ingested microfilariae, and that of mosquitoes dissected 8.5 to 12.5 days later, 24 percent were infected. Practically all larvae were stage III (infective) in mosquitoes dissected 12.5 days after feeding. In other observations with *M. longipalpis*, 26 percent (11 of 42) were infected 10.5 days after feeding, and infective larvae were found in 7 percent (3 of 42). Of all larvae recovered from these mosquitoes, 40 percent (34 of 84) were stage III.

Development of *D. magnilarvatum* and *W. malayi* sometimes took place in the same mosquito. One *M. longipalpis* dissected after 12.5 days contained 31 *W. malayi* larvae, 23 of which were in the head, and 6 *D. magnilarvatum* larvae, 4 of which were in the head. Stage III larvae were found in mosquitoes identified as *M. longipalpis* and *M. bonneae*. No larval chitinization was observed, but abnormal larvae were occasionally found.

Of the *M. uniformis* examined 7.5 to 13.5 days after feeding, 21 percent were infected. Although only 2 infective larvae were found in 1 mosquito, this species also appears to be a suitable laboratory host for *D. magnilarvatum*, since development proceeded to the infective stage in from 12.5 to 13.5 days. Most of the mosquitoes were dissected 9.5 to 10.5 days after feeding, at which time nearly all the *W. malayi* larvae were stage III, while most of the *D. magnilarvatum* larvae recovered were only stage I or II, though some were nearing maturity and were 800 microns in length. Stage III larvae were found in the head of a mosquito kept for 13.5 days before dissection, indicating that *D. magnilarvatum* may require more time to develop in *M. uniformis* than does *W. malayi*.

Development of *D. magnilarvatum* to the infective stage has been observed also in *M. annulatus* (2 of 11 mosquitoes dissected) 13.0 days after feeding. No infections were seen in 27 *Aedes aegypti* and 26 *A. albopictus* which fed on an infected monkey and were dissected 15 to 16 days later.

Appearance of Infective Larva

Stage III larvae of *D. magnilarvatum* could be easily differentiated from those of *W. malayi*. They could be distinguished in saline when freshly dissected from the mosquitoes on the basis of movement, which for *D. magnilarvatum* was slow and writhing and for *W. malayi* rapid and wriggling. A summary of the measurements of stage III larvae of *D. magnilarvatum* is given in table III. When compared with

TABLE III. *Showing the measurements of stage III larvae of* D. magnilarvatum *in microns.*

Specimen number	Length	Width	Nerve ring	Oesophagus	Anus to tail	Anal ratio
1	1035	24	90	375	41	2.0
2	1050	26	86	405	34	2.0
3	1005	24	90	390	38	2.2
4	1005	24	90	405	38	2.2
5	1095	24	90	375	41	2.2
6	1020	26	86	–	38	2.2
7	960	25	–	–	34	2.0
8	1005	26	90	390	34	2.0
9	1050	26	–	–	38	2.2
Mean	1025	25	89	390	37	2.1

the measurements of stage III larvae of *W. malayi*, it is found that those of *D. magnilarvatum* are shorter, have more rounded tails, and have the anal opening

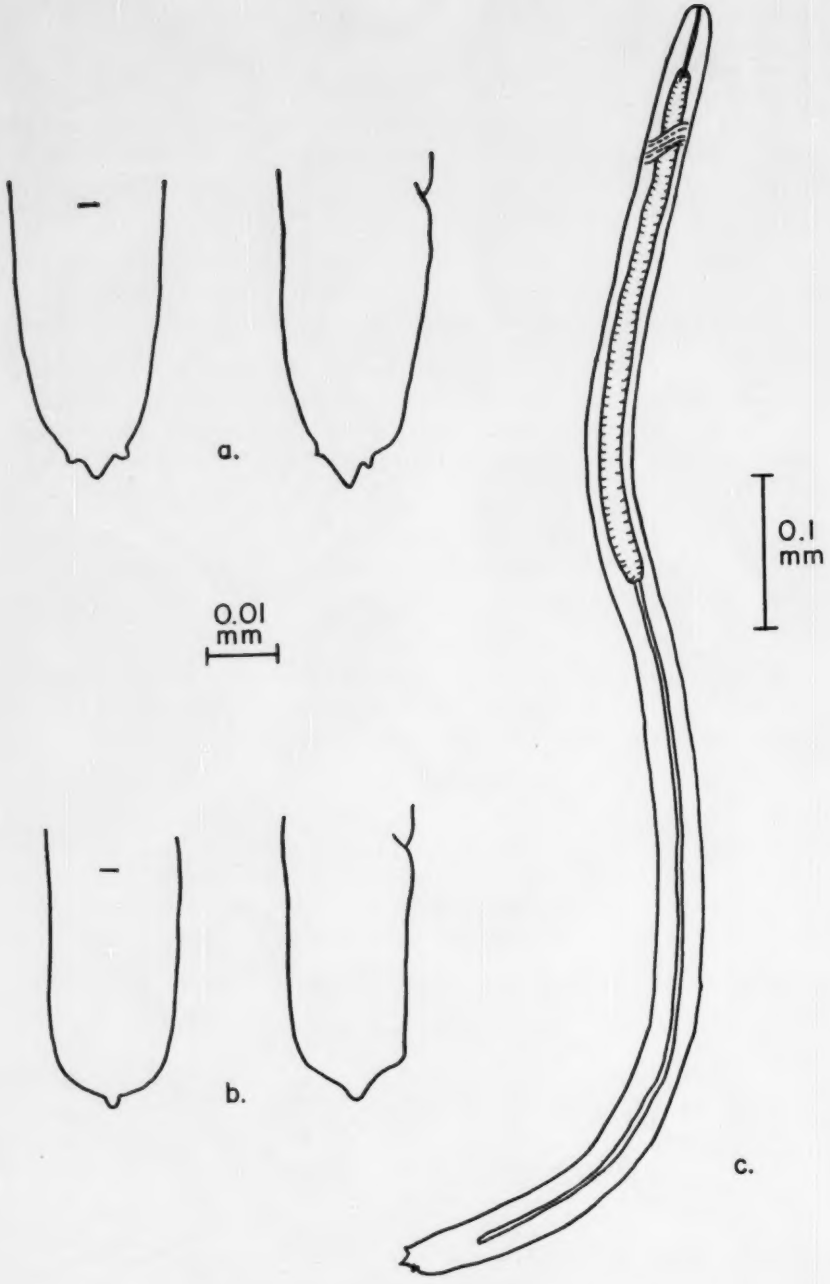
a.
0.01
mm
b.
0.1
mm
c.

FIGURE 1. The appearance of stage III larvae of *Dirofilaria magnilarvatum* with the terminal region of *D. immitis* larvae for comparison. a. Terminal region of *Dirofilaria magnilarvatum:* ventral and lateral view. b. Terminal region of *Dirofilaria immitis:* ventral and lateral view. c. Entire larvae *Dirofilaria magnilarvatum.*

closer to the end of the tail. Their length varied from 960 to 1095 microns compared with 1235 to 2000 microns for *W. malayi* (Wharton, 1957) and 1335 to 1725 microns for 8 *W. malayi* larvae which had developed in the same mosquitoes. The anal ratio was 2.1, range 2.0 to 2.2, which is about the same as in *D. immitis,* but much smaller than the ratio of 4.1, range 3.3 to 4.5 recorded for *W. malayi* larvae by Wharton.

Although *D. magnilarvatum* and *D. immitis* larvae are very similar, they could be distinguished by the form of the terminal papillae (fig. 1). In *D. magnilarvatum* the terminal papilla is pointed and flanked on either side by a small secondary pointed papilla, whereas in *D. immitis* there is usually only 1 blunt papilla. (According to Iyengar, 1957, there is occasionally a second small papilla behind the tip.) These papillae would never be confused with those of *W. malayi* or *W. bancrofti,* since they are strictly terminal and pointed, projecting beyond the rounded tip of the tail.

SUMMARY

The development of large microfilariae from the long-tailed macaque (*Macaca irus*), later described as *Dirofilaria magnilarvatum* Price, was observed in *Mansonia* (*Mansonioides*) *longipalpis* and *M. uniformis.* A monkey infected with both *D. magnilarvatum* and an infection resembling *Wuchereria malayi* was used in most experiments.

Microfilariae of *D. magnilarvatum* were ingested by *M. longipalpis* and *M. uniformis,* but the number ingested was not related to the estimated microfilarial density in the blood as in the case of *W. malayi.*

Development of the larvae took place in the Malpighian tubules. In *M. longipalpis* some stage III larvae were found 10.5 days after the infecting blood meal, and most larvae were stage III by 12.5 days. The development of larvae of *D. magnilarvatum* was slightly slower in *M. uniformis* than in *M. longipalpis* in that no stage III larvae were present in the former mosquitoes examined 10.5 days after the infecting blood meal. Microfilaria of *W. malayi* were nearly all stage III after 10.5 days in *M. uniformis.*

The stage III larvae resembled *D. immitis,* the only difference observed being in the form of the terminal papillae. Stage III larvae were 960 to 1095 microns in length, much shorter than *W. malayi* larvae in the same mosquitoes which were 1335 to 1725 microns.

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RESEARCH NOTE

SIGNIFICANCE OF HUMAN INTESTINAL HELMINTHS IN EASTERN KENTUCKY.

Prevalences of *Ascaris lumbricoides* and *Trichuris trichiura* were determined at rates of approximately 21 and 15 percent, respectively, in Floyd and Letcher Counties, Kentucky by Atchley, Hemphill and Hunt (1956, J. Parasit. **42**: 505–509). During the time that the studies were made on the normal population, additional parasitological observations were carried out, through fecal examination, on 1,244 patients admitted for all causes to one hospital in Floyd County. Roundworm and whipworm infections were noted far more frequently than those of any other intestinal parasites, as was true in the general population. The hospital study also involved consideration of *Strongyloides stercoralis* and hookworm.

Thirty-two percent of the patients of all ages, who were examined from March 1955 through December 1956, were observed to be infected with one or more species of helminths. Results were based on examination of a single stool at admission. The parasites diagnosed with percent positive follow: *Ascaris* (12), *Trichuris* (14), *Strongyloides* (5), and hookworm (1). No monthly or seasonal trends were discernible.

Subsequent examinations of the hospital records were made to ascertain whether or not the attending physicians had included clinical indications of helminthiasis in the final diagnoses. It was discovered that such references had been made in 7 percent of the cases. In 2 percent of the 1,244 patients, *Ascaris* was implicated; *Trichuris*, in 1 percent. The records also indicated effects of a *Strongyloides* infection as being significant in a single case, while in 4 percent the particular parasite responsible was not stated.

Instances of more serious consequences such as intestinal obstruction brought about by infection with *Ascaris* were reported to be a familiar occurrence at the hospital. In the period of the investigation, the death of a 4-year-old child residing in the area was found at autopsy to have been caused by a heavy infestation of *Trichuris*. Surgical complications of ascariasis were quite generally well known to physicians locally and in neighboring regions (J. B. Floyd, April 1957, Am. Acad. Gen. Practice, Ky. Ch., Ann. Meeting, Louisville, Ky.).

An apparently indifferent attitude among the populace relative to infections with intestinal worms predominated in the area. Accompanying factors included the commonplaceness of helminthic occurrences and a lack of awareness as to their more occult effects. It was only when children less than approximately 2 years of age were infected that some concern was ordinarily evident. Observations on the normal population disclosed as high as 19 percent of 162 children examined in this age group to be harboring *Ascaris*. Although a specific study was not made to determine the prevalence of *Enterobius vermicularis*, incidental observations strongly indicated ubiquitous distribution in the area for the parasite and its obvious symptoms.—F. O. ATCHLEY, *Communicable Disease Center, U. S. Department of Health, Education, and Welfare, Bureau of State Services, Public Health Service, Phoenix, Arizona.*

THE CELLULAR RESPONSE OF WHITE RATS TO *LITOMOSOIDES CARINII* LARVAE AS INFLUENCED BY CORTISONE, AGE, AND PREVIOUS INFECTION.*

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Previous experiments have established that in old white rats (over one year of age) an effective resistance exists or develops against the successful migration of infective *Litomosoides carinii* larvae (Olson et al, 1954, 1958a). Only 2 percent of these larvae completed migration from skin to pleural cavity as compared to an average of 42 percent in cotton rats (normal host). When the resistance of white rats to migrating larvae was bypassed by surgical transfer of post-migratory stages from cotton rats to peritoneal cavities of white rats, worm survival was significantly increased. It was, however, noted that encapsulation of transferred worms by host cells was more commonly observed in white rats than in cotton rat controls.

To account for resistance to migrating larvae and encapsulation of post-migratory stages, it was proposed that white rats offer a greater cellular response to this infection than do cotton rats. These experiments were also designed to compare the cellular responses of young and old rats to this infection. A portion of these results has been reported in abstract (Olson, 1958b).

METHODS AND MATERIALS

White rats (Carworth-Wistar), cotton rats (*Sigmodon hispidus texianus*), worms (*L. carinii*, Eastern strain), and mites (*Ornithonyssus bacoti*) were obtained and treated as described by Olson (1958a). Rats were infected subcutaneously with known numbers of 3rd-stage larvae recovered from mites which had fed previously on infected cotton rats (Macdonald and Scott, 1953). Second infections were similarly given in the flank opposite the first infection. Young rats of known age were obtained from breeding stock in the laboratory. Cortisone acetate (Schering) was injected subcutaneously at 30 mg per kg body weight. An equal volume of sterile physiological saline was similarly given to each control rat. Higher doses of cortisone were found to cause excessive weight loss in rats. When cell counts were made from pleural cavity exudates, the method of Bloom et al (1950) was modified for use in pleural cavities. Tyrode's solution (25 ml per kg body weight) was injected in equal volumes into the left and right pleural cavities. Samples of the diluted exudate were taken from each cavity for total and differential white cell counts. Statistical analysis was based on methods from Snedecor (1946). When percentages based on less than 100 items were used, a transformation was done to compensate for the sample size (Snedecor, 1946, p. 449). Probability levels greater than 5 percent were considered as having no statistical significance.

EXPERIMENTAL PROCEDURES AND RESULTS

Design of Experiment 1. The experiment was designed to test the survival of worms in white and cotton rats within a 10-day period of infection, since previous experiments had indicated this to be the crucial period in the survival of th parasite, i.e., the larval migration phase from skin to pleural cavity (Olson, 1958a). Rats of various ages were included to reveal

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differences in resistance between immature and mature rats. Within each of 7 age groups, 5 types of rats were tested: (1) WR-C, infected white rats injected with cortisone; (2) WR-S, infected white rats injected with saline; (3) CR-S, infected cotton rats injected with saline; (4) WR, uninfected white rats; (5) CR, uninfected cotton rats. All infected rats at each age received larvae from the same pool of mites. Daily cortisone and saline injections were begun 5 days prior to infection and continued throughout the 10 days of infection. Replicate rats within each type were given nearly equal numbers of infective larvae (table I).

TABLE I. *Data on pleural cavity exudate from 10-day infections: (1) mean total white cell count in thousands per cu mm; (2) mean cells per worm; (3) mean percentage eosinophils; (4) mean number infective larvae.*

Type of rat		Age of rats in weeks						
		2	4	6	8	11	14	28
White rats, cortisone, infected	(1)	1	2	1	3	2	2	2
	(2)	−28	−3	−30	16	−7	−5	8
	(3)	1	3	1	5	5	4	5
	(4)	55	146	71	137	84	118	96
White rats, saline, infected	(1)	2	6	7	15	6	5	7
	(2)	12	27	97	194	94	50	267
	(3)	21	31	42	30	38	15	39
	(4)	51	140	75	130	78	124	102
Cotton rats, saline, infected	(1)	8	27	28	28	6	11	9
	(2)	92	217	466	293	51	84	80
	(3)	19	43	36	44	50	22	58
	(4)	62	105	76	109	71	83	84
White rats, uninfected	(1)	2	1	2	2	3	2	2
	(3)	1	4	3	5	3	2	5
Cotton rats, uninfected	(1)	4		3	4	4	2	2
	(3)	5		5	2	1	1	2

Pleural Cavity Exudates. At autopsy total white cell counts from pleural cavity exudates showed that both species of rats responded to this infection with increased numbers of cells. This response was suppressed in WR-C. Cell counts did not increase or decrease significantly with age or number of infective larvae in any of the types of rats. It seemed that a more accurate relationship between the cellular response and infection would be obtained by relating cell counts to the number of worms present in the pleural cavity at autopsy.

To show only the increase in total cells which resulted from infection, the average number of cells present in uninfected rats was subtracted from the number of cells present in each infected rat of the same species. This increase in total cells was divided by the number of worms present to obtain the number of inflammatory cells per worm. In cortisone-treated animals these numbers were negative if total cell counts were below the mean for uninfected rats. It should be noted that these counts were based on diluted samples, and represent a fraction of the actual number of cells (table I). The mean number of cells per worm for CR-S was 183 ± 217 as compared to 106 ± 102 for WR-S, and -7 ± 16 for WR-C. When cells per worm were plotted against age groups and a regression line fitted to the data, no significant increase or decrease in the cellular response was noted for older rats. It was concluded that the inflammatory response in the pleural cavity, as measured by these methods on the tenth day of infection, did not significantly change with the age of the host, nor was the variation in cell counts explained by the number of worms which had completed migration.

Differential cell counts were also made from samples of pleural cavity exudate (table I). A striking increase in the eosinophil count was noted in WR-S and CR-S, averaging 31 and 39 percent, respectively. The injection of cortisone suppressed the eosinophil response of WR-C to levels near those of uninfected rats, averaging

about 3 percent for each type of rat. No significant changes in eosinophil counts were noted with increased age in any type of rat. The other cells in infected rats treated with saline were primarily members of the monocytic series, i.e., lymphocytes, monocytes, polyblasts, and macrophages. Only an occasional heterophil was seen. The other types of rats had similar cell populations but in different propor-

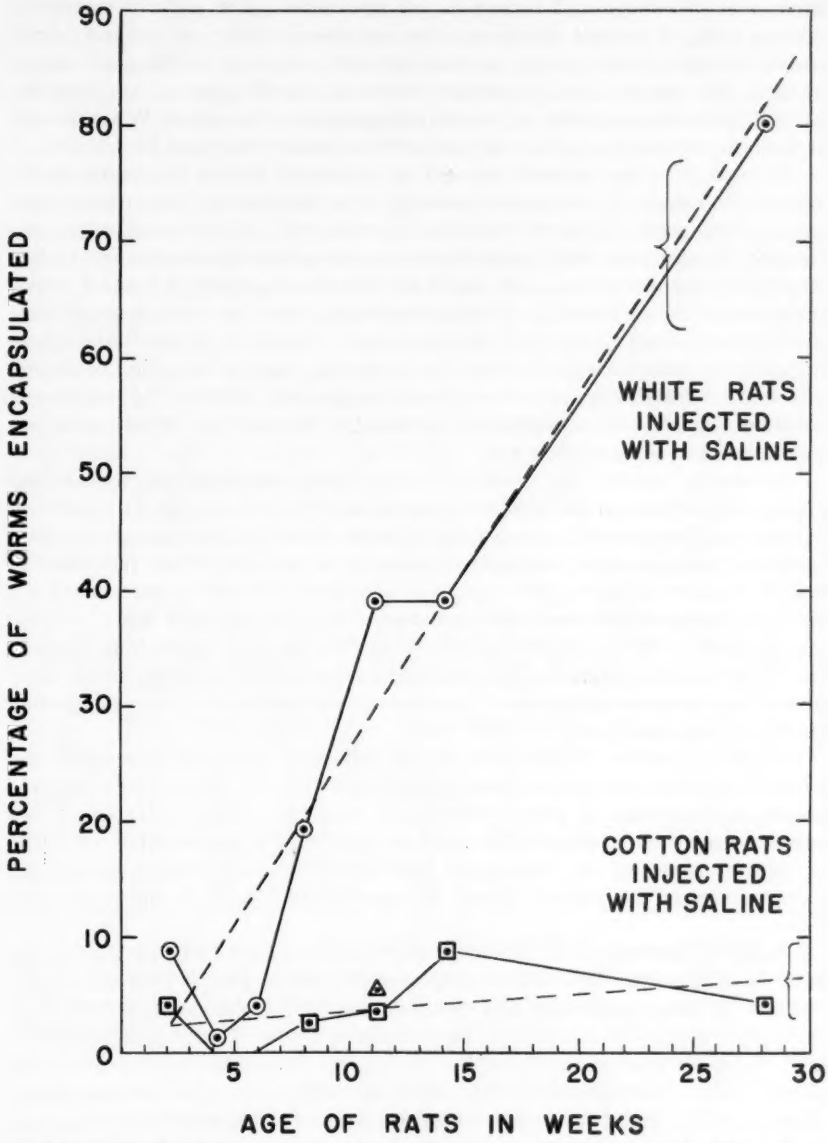


FIGURE 1. Percentages of encapsulation for larvae recovered from pleural cavities of rats infected for 10 days.

tions, i.e., fewer eosinophils.

Encapsulation. The number of worms with definite evidence of encapsulation by host cells was expressed as a percentage of total worms present (fig. 1). Many encapsulated larvae were still alive as evidenced by movement, others were quiescent and of doubtful viability. Worms recovered from cotton rats showed little encapsulation with an average of 3 percent for all ages tested. The slope of regression (shown in fig. 1) was not significant. Encapsulation in WR-S, at the first 3 ages tested, was approximately within the range for CR-S; however, in WR-S of 8 weeks or older, the encapsulation percentages were significantly greater than those in CR-S. Cortisone completely suppressed encapsulation of worms in WR-C except at the 11th week when a percentage was obtained within the range for CR-S.

No correlation was apparent between the number of cells in the pleural cavity and the percentages of encapsulated worms. The inflammatory responses of cotton rats were generally greater than those in white rats, yet encapsulation was infrequent in cotton rats. Also, encapsulation was not necessarily accompanied by the larger cell counts per worm. Cell counts for WR-S were greater at 6 and 8 weeks of age than at 11 and 14 weeks, yet encapsulation was less in the younger age groups. Similar observations may be made for cotton rats. Admittedly, host cells must participate in the encapsulation of worms, but apparently high cell counts alone do not explain the increased encapsulation of worms in the older WR-S. This conclusion is further supported by no significant increase (or decrease) in the cell count per worm in either species of older rat.

Survival of Larvae. The number of larvae which completed migration to the pleural cavity was expressed as a percentage of the infective dose (fig. 2). Analysis of regression lines plotted for percentages of worm recovery against age groups indicated that worm survival decreased significantly in the older white rats, but remained constant in older cotton rats. No significant differences were found, by analysis of variance, between worm recoveries in WR-S and those in WR-C. Thus, the decreased recoveries in older white rats does not appear to result from destruction of worms after migration into the pleural cavity, since cortisone almost completely suppressed the inflammatory response and encapsulation of worms in pleural cavities of both young and old white rats.

Length of Larvae. Worms free of host cells were measured to compare the effects of different host environments upon growth (fig. 3). Worms grew slightly longer, on the average, in cotton rats than in white rats; however, analysis of covariance showed no significant differences in mean lengths between white rat types or between species of rat. Regression lines plotted for mean lengths against age groups confirmed the constant growth of worms in all 3 types of rats at the ages tested.

Design of Experiment 2. The experiment above demonstrated an "age resistance" in mature white rats which was expressed in two ways: (1) decreased percentages of larvae completing migration from skin to pleural cavity, and (2) increased percentages of encapsulated post-migratory forms. Cortisone did not suppress resistance to migrating larvae, but did suppress encapsulation of worms in the pleural cavity. The higher percentages of encapsulated worms were not consistently accompanied by higher cell counts, but appeared to be associated with the onset of maturity of the white rat. The possibility was considered that acquired resistance had developed and influenced the encapsulation of worms in mature white rats. It

was proposed that a higher percentage of worms would be encapsulated in immunized rats than in non-immunized rats, and that mature rats would more rapidly acquire resistance to this infection than would immature rats.

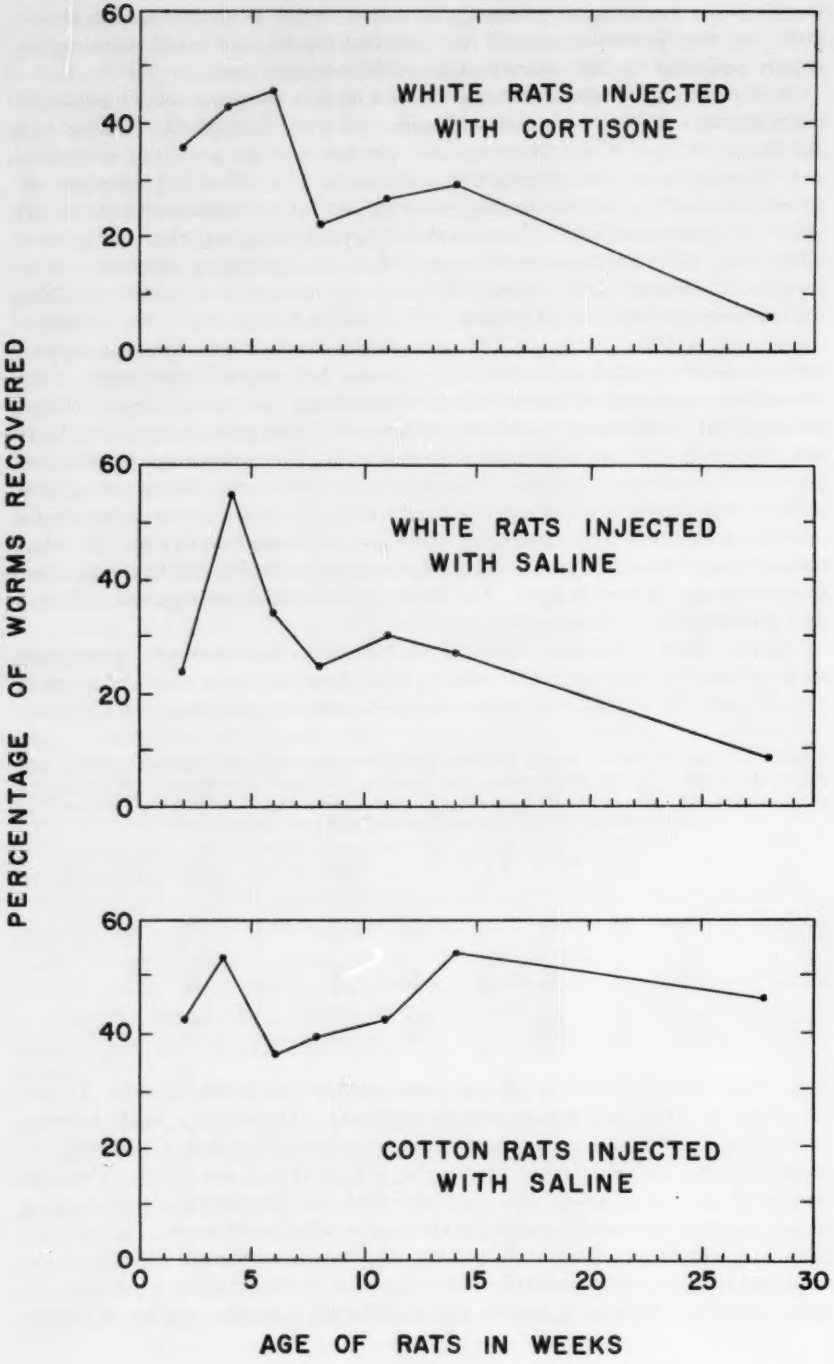
Rats of two ages were in the experimental design: immature rats, 3 weeks old when immunized; and mature rats, 8 weeks old when immunized. Within each age group, 10 types of rats (two replicates per type) were tested: (1) immunized and challenged white rats injected with cortisone, or (2) saline; (3) white rats infected as controls to the immunizing infections and injected with cortisone, or (4) saline; (5) cotton rats infected as controls to the immunizing infections and injected with saline; (6) white rats infected as controls to the challenging infections and injected with cortisone, or (7) saline; (8) cotton rats infected as controls to challenging infections and injected with saline; (9) uninfected white rats; (10) uninfected cotton rats. (WR-C, WR-S, CR-S: abbreviations in text for white rats injected with cortisone or saline, and cotton rats injected with saline, respectively). The immunizing worms and their controls were introduced into rats on day 0 (70 ± 10 larvae per rat); challenging worms and their controls were given on day 21 (75 ± 10 larvae per rat). All rats were autopsied on day 31. Rats at each age received larvae from the same pool of mites. Daily injections of cortisone (30 mg per kg) and saline for immunized rats and controls were started on day 0; similar injections for rats infected as controls to challenging infections were started on day 16. All injections continued through day 31. Rats were weighed every 7th day to adjust injections to changes in body weight. The 10-day control infections supplied additional data for the previous experiment.

Pleural Cavity Exudates. Total white cell counts and eosinophil percentages were increased in both species of infected rats; these responses were depressed in WR-C (table II). Cells other than eosinophils were overwhelmingly of the mono-

TABLE II. *Data on pleural cavity exudates for immunizing and challenging infections: (1) mean total white cell count in thousands per cu mm; (2) mean percentage eosinophils; (3) mean number cells per worm (WR, white rat; CR, cotton rat; S, saline; C, cortisone; 31 immunizing or control worms, 10, challenging or control worms).*

Age group	WR–S 31–10	WR–C 31–10	WR–S 31	WR–C 31	CR–S 31	WR–S 10	WR–C 10	CR–S 10	WR (uninfected)	CR (uninfected)
(1)										
Immature	30	4	25	2	32	7	1	28	2	3
Mature	38	5	7	5	18	6	2	6	3	4
(2)										
Immature	26	3	22	3	41	42	1	36	3	5
Mature	46	10	44	15	51	38	5	50	3	1
(3)										
Immature	323	21	429	1	663	102	−21	468		
Mature	1079	37	542	65	183	82	−22	60		

cytic series. No difference in cell types was noted in rats harboring either 31-day-old worms or 10-day-old worms, or both infections. Apparently a single infection of 31 days duration was a greater stimulus to inflammation than a single 10-day infection. This observation may be made for WR-S, WR-C, and CR-S. Thus, the number of cells per worm in white rats with both the immunizing and challenging worms appeared to depend more on the effect of the 31-day-old worms. If one compares, however, the number of cells per worm in immunized mature WR-S with the combined responses in the respective control rats, it is seen that the combination of immunizing and challenging worms acts to stimulate a greater number of inflam-


WHITE RATS INJECTED WITH CORTISONE
WHITE RATS INJECTED WITH SALINE
COTTON RATS INJECTED WITH SALINE
PERCENTAGE OF WORMS RECOVERED
AGE OF RATS IN WEEKS
0
20
40
60
0
5
10
15
20
25
30

matory cells per worm than the sum of these stimuli acting in separate but similar animals.

Encapsulation. The percentages of challenging worms (and their controls) which were encapsulated were recorded at autopsy (table III). It was concluded

TABLE III. *Data on worms from challenging and control infections: (1) mean percentage of encapsulation; (2) mean percentage of recovery; (3) mean length in mm.*

Age group	White rat saline	White rat cortisone	White rat saline	White rat cortisone	Cotton rat saline
	CHALLENGING WORMS		CONTROL WORMS		
(1)					
Immature	11	0	4	0	0
Mature	64	11	38	5	5
(2)					
Immature	19	41	34	36	37
Mature	6	29	30	25	42
(3)					
Immature	0.97	0.95	0.98	0.87	1.23
Mature	1.02	0.91	0.98	0.92	0.95

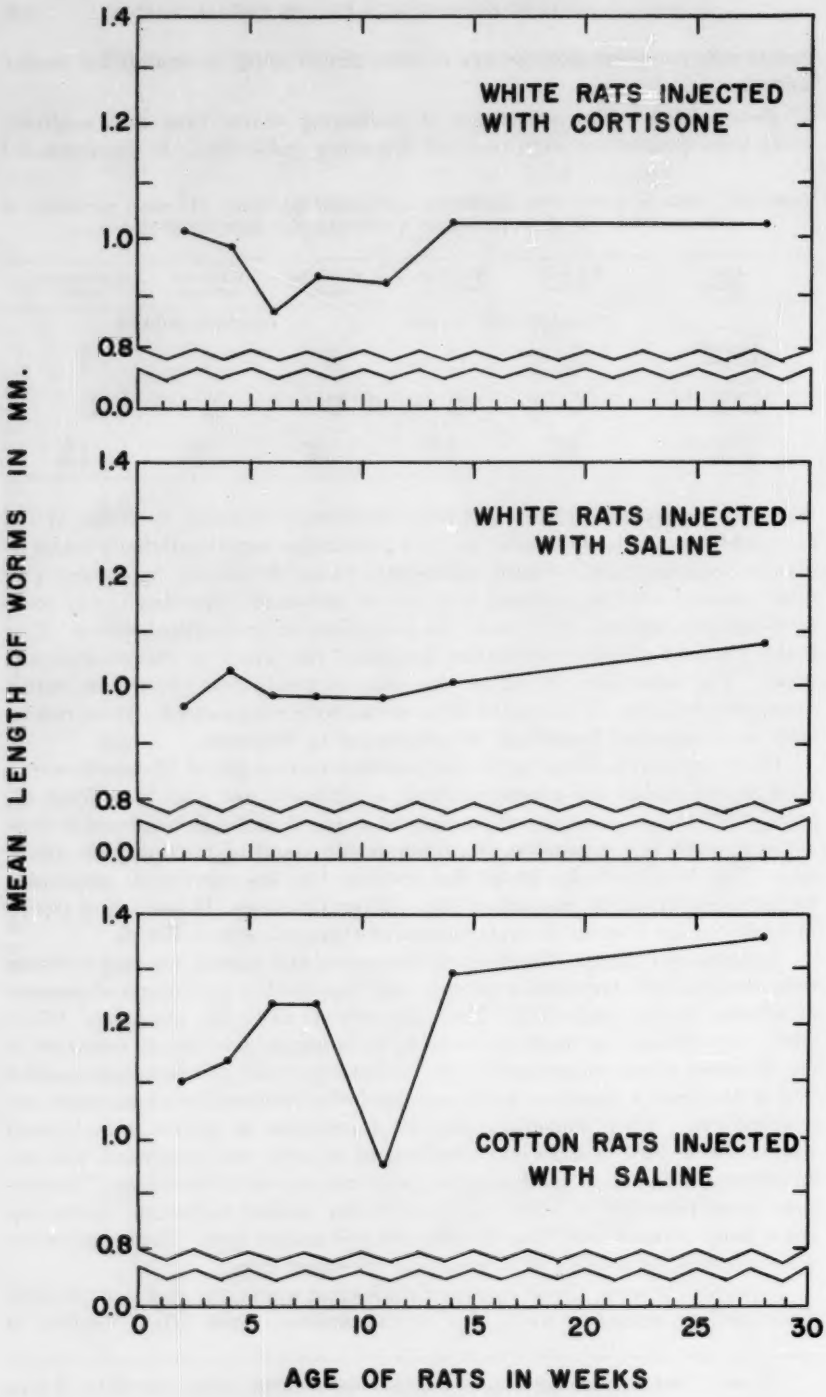
that: (1) encapsulation percentages were significantly increased in mature WR-S as compared to similar immature rats; (2) percentages were significantly higher in mature immunized WR-S than in control rats, 64 and 38 percent, respectively (the same tendency noted in immature rats was not statistically significant); (3) cortisone injections significantly reduced the percentages of encapsulated worms. Cortisone was most effective in immature immunized rats where no encapsulation was noted. The same dose (30 mg per kg) was apparently less effective in mature immunized rats since 11 percent of these worms were encapsulated. These conclusions were supported by analysis of variance and by regression.

In the previous experiment, the encapsulation percentages of 10-day-old worms were plotted against age groups to obtain a regression line (fig. 1). When the value of 64 percent (cited above) was plotted at age 11 weeks, it was found to deviate significantly from regression, i.e., within the fifth standard deviation from regression. The third conclusion stated that cortisone was less effective in suppressing encapsulation in mature immunized rats. When this value, 11 percent, is plotted in figure 1 at age 5 weeks, it can be seen as an expected value of WR-S.

Survival of Worms. Numbers of challenging and control worms (including those encapsulated) recovered at autopsy were expressed as percentages of numbers of infective larvae (table III). Thes data indicate that: (1) immunized WR-S offer a significantly increased resistance to a challenging infection as compared to the resistance of non-immunized rats to control infections; (2) mature immunized WR-S developed a resistance which was more effective than that of immature immunized rats. When immunized rats, either immature or mature, were injected with cortisone, their resistance to a challenging infection was suppressed, and was no different from that of non-immunized white rats to control infections. The constant worm recoveries in cotton rats showed that viability to infective larvae was not a factor between infections of immature and mature rats. These conclusions were supported by analysis of variance and chi-square tests.

Lengths of Worms. Mean lengths of challenging worms and their controls were determined by measuring worms free of encapsulation (table III). Analysis of

FIGURE 2. Percentages of larvae recovered from pleural cavities of rats infected for 10 days.


WHITE RATS INJECTED WITH CORTISONE
WHITE RATS INJECTED WITH SALINE
COTTON RATS INJECTED WITH SALINE
MEAN LENGTH OF WORMS IN MM.
AGE OF RATS IN WEEKS
1.4
1.2
1.0
0.8
0.0
0
5
10
15
20
25
30

covariance was used to test for significant differences between mean lengths of worms. The factors of age, previous immunization, and cortisone injection did not significantly influence the mean lengths of 10-day-old worms. An attempt was made to compare mean lengths of encapsulated and non-encapsulated worms. Most of the encapsulated worms were not suitable for measurement due to the presence of host cells. Twenty encapsulated worms from mature white rats were measured, however, and found to have a mean length of 0.71 mm, which was not significantly different from the mean of 0.96 mm for non-encapsulated worms from similar rats. Similar comparisons were not made for worms from immature rats since very little encapsulation occurred in that age group.

Cellular Composition of Capsule. Several encapsulated worms recovered from white rats were stained as whole mounts with Wright's stain. In other preparations the capsule was spread on a slide and stained. The cell types in capsules of 10-day-old worms were not noticeably different from those of 31-day-old worms. The most striking observation was the large number of eosinophils within the capsule or on its periphery. Some eosinophils appeared to be directly adjacent to the surface of the worm. The other cells were primarily macrophages, monocytes, and fibroblasts. The cells were located in a fibrinous matrix which appeared condensed at the periphery of the capsule and on the surface of the worm. The morphology of some worms was easily recognizable, other worms had apparently undergone considerable digestion with primarily cuticle and brownish-yellow pigment granules remaining. The cellular composition of these capsules resembled that described by Wharton (1947) in cotton rats. In the 31-day infections, it was observed that encapsulation of shed cuticles also occurred.

Experiment 2 also allowed the following observations on the survival and development of worms during a 31-day period of infection, i.e., the immunizing and control worms.

Encapsulation. Encapsulation percentages tended to be higher in mature than in immature white rats (table IV). It also appeared that cortisone acted to depress

TABLE IV. *Data on worms from immunizing and control infections: (1) mean percentage of encapsulation; (2) mean percentage of recovery; (3) mean length of male (M) and female (F) worms in mm; (4) mean percentage molting into or in adult stage.*

Age group	White rat saline		White rat cortisone		White rat saline		White rat cortisone		Cotton rat saline	
	IMMUNIZING WORMS				CONTROL WORMS					
(1)										
Immature	5		0		0		0		0	
Mature	14		9		33		2		1	
(2)										
Immature	50		53		47		60		40	
Mature	15		19		6		28		48	
(3)	M	F	M	F	M	F	M	F	M	F
Immature	15	21	13	18	13	16	16	23	15	20
Mature	8	11	9	10	11	8	9	11	14	15
(4)										
Immature	100	100	100	100	100	92	100	97	100	100
Mature	100	91	86	65	100	100	91	88	100	87

the frequency of encapsulation. Analysis of variance showed, however, no significant differences between age groups of white rats, or between WR-S and WR-C, or

FIGURE 3. Mean lengths of larvae recovered from pleural cavities of rats infected for 10 days.

between immunizing worms and their controls. The higher percentages, again, did not ncessarily accompany the higher cell counts per worm.

Survival of Worms. Based on analysis of variance, it was concluded that 31-day worms survived as well in immature white rats as in cotton rats of either age (table IV). Mature white rats offered a significantly greater resistance to worm survival than did immature rats. Although a higher percentage of worms survived in WR-C, this difference was not significant as compared to recoveries in WR-S. It was also found that no significant differences existed between the survival of immunizing worms and their controls. Male worms survived as well as female worms in any of the types or age groups of rats. The recoveries in both groups of cotton rats indicated that viability of infective larvae was not a factor in the decreased survival of worms in mature white rats.

Length and Development of Worms. The mean lengths of 31-day worms (except encapsulated worms) were compared by analysis of covariance and "t" tests (table IV). Lengths of worms were less in mature than in immature white rats, regardless of treatment. This difference was, however, significant only for female worms. Worm growth was decreased in mature white rats, but worms in immature white rats grew as well as in cotton rats of either age. The observations on morphological development, in general, support those for growth (table IV). Worms of either sex developed similarly in immature white and cotton rats. Female worms showed a slightly retarded development in the mature rats. Analysis of variance showed no significant differences for comparisons between ages, treatments, or types of rat.

DISCUSSION

In these experiments, cortisone effectively depressed the cellular response in the pleural cavities of white rats infected with *L. carinii* to levels approximating those in uninfected rats. In 10-day infections, the percentages of larvae which completed migration in WR-C were not, however, greater than those in WR-S. Apparently, the greater resistance of previously unexposed white rats (as compared to cotton rats) to migrating larvae is not explained by a superior capacity to mobilize an inflammatory response to this infection. It should be noted that the cellular response in the pleural cavities of cotton rats was greater than that for white rats; yet, cotton rats are significantly less resistant to this infection.

An "age resistance" was noted in mature white rats, while the resistance of cotton rats remained consistently low at all ages tested. Age resistance was evidenced by decreased percentages of larvae completing migration, and increased percentages of encapsulated larvae. The cellular response in the pleural cavities of white rats could not be correlated with development of age resistance. Cotton rats had higher average cell counts than white rats, but had very low percentages of encapsulated worms. Since the first experimental evidence by Looss in 1911 that age is a factor in host resistance to a nematode infection, several investigators have made similar observations for a number of host-nematode relationships (Ackert, 1942). Sandground (1929) held the view that the young of related species of hosts have common physiological and anatomical characteristics, but as these related species grow older, physiological and anatomical differences develop; hence, a parasite adapted to a particular species of host would find its requirements most closely met in the young of a related species of host. Chandler (1955, p. 26) suggested that in normal hosts

a series of influences or stimuli guide the majority of migrating larvae properly toward their normal location in the body of the host. In abnormal or strange hosts, these guiding influences are either absent or misleading; hence, a considerable number of migrating larvae do not reach their normal location, but migrate into unusual areas of the body. In the case of *L. carinii,* cotton rats and immature white rats represent environments similar enough so that migrating larvae are able to penetrate tissues and arrive in pleural cavities of either host with equal success. In mature white rats the environment has apparently changed with the result that many larvae do not complete migration to the pleural cavities within the 10-day period in experiment 1 (or 31 days in the case of experiment 2). Lewert and Lee (1954) provided a possible explanation for age resistance to migrating nematode larvae. These workers have shown by histochemical techniques that larvae cannot penetrate through the connective tissues of old white rats as readily as in younger rats. In the case of *L. carinii,* the route of migration is not fully known; however, connective tissue must be encountered by migrating larvae in at least two areas, i.e., subcutaneous and pleural. Recent unpublished work with *L. carinii* and cortisone-treated white rats indicates that larval migration may be significantly delayed in older animals (10 – 14 months) (Briggs, 1958).

Acquired resistance to this infection has been shown by Macdonald and Scott (1953) in immunized cotton rats. They have also shown that sera recovered from infected cotton rats react with cultured *L. carinii* larvae to form precipitates similar to that described by Sarles (1938) and other workers. Briggs (1955) has shown a similar precipitate formation with sera from infected white rats. About 2 weeks are required following the infection of a white rat to obtain sera which will react with cultured *L. carinii* to form detectable precipitates (Briggs, 1958). There is also evidence that the rat can develop detectable antibody to a single antigenic dose within a 10-day period (Berglund, 1956). In mice, Larsh et al (1952) have shown that effective acquired resistance to an initial infection of *Trichinella spiralis* is developed by the 11th day. It is generally agreed that mature animals are more capable of producing antibody than immature animals (Culbertson, 1941; Raffel, 1953). In the case of mature white rats, it seems doubtful that acquired resistance played a significant role in the decreased survival of migrating larvae as measured on the 10th day by recoveries in pleural cavities, since cortisone injections did not increase the percentages of larvae completing migration. Several workers have shown that this compound can decidedly depress antibody levels in various animals, including the rat (Bjorneboe, 1951; Dews and Code, 1953; Newsom and Darrack, 1956; Dixon, 1954; Berglund, 1956; Germuth, 1956). Since daily cortisone injections were started 5 days before infection, and were continued during the 10 days of infection, it seems that antibody production would have been depressed. Worm survival was not, however, significantly increased (or decreased) in WR-C at any of the ages tested as compared to worm survival in similar WR-S. This finding suggests that antibodies do not reach effective tissue levels before the larvae have completed their migration in mature white rats.

The results of the last experiment showed that encapsulation of challenging worms was significantly increased in mature immunized white rats as compared to immature immunized rats. Apparently, immunization promotes encapsulation of worms, and does so more effectively in mature rats than in immature rats. As also

noted in experiment 1, the number of inflammatory cells per worm did not correlate with the percentages of encapsulation, although the cellular response of mature immunized WR-S was comparatively very heavy. This increased response of immunized animals to challenging infections has been noted for other host-nematode relationships (Chandler, 1937; Kerr, 1938; Taliaferro and Sarles, 1939, 1942; Cort and Otto, 1940; Larsh and Race, 1954). On the basis of these results it is suggested that the increased encapsulation of worms noted in mature white rats in experiment one, was due to an acquired resistance which developed during the 10 days of primary infection, and acted to facilitate the encapsulation of post-migratory larvae by host cells in the pleural cavities.

It was also noted in the last experiment that the percentages of challenging larvae which completed migration to the pleural cavities of white rats were significantly decreased. This decrease was most apparent in the mature age group. Apparently immunization also increases the resistance of white rats to migrating larvae. Cortisone treatment suppressed the resistance of immunized white rats to migrating larvae; cortisone did not, however, suppress "age resistance," i.e., the decrease in worm recoveries noted in the first experiment with older white rats. The effective suppression of acquired resistance in immunized and challenged rats by cortisone is evidence that in experiment one the same dosage of the hormone (30 mg per kg) was also effective in suppressing any acquired resistance which may have developed in white rats exposed to only a single infection of 10 days duration.

The increased resistance of immunized animals to nematodes has been explained by several workers as depending primarily upon the action of protective antibodies with secondary cellular cooperation (Larsh and Race, 1954). Other workers have appeared to emphasize the role played by the cellular response of immunized animals to invading nematodes (Coker, 1956). To evaluate the relative importance of phagocytes and antibodies in host resistance is difficult. In addition to the interaction between antibodies and inflammatory cells, the problem of determining protective levels of these components must be solved for the particular host-nematode relationship. Furthermore, comparisons between experiments with cortisone-treated hosts and nematode infections are complicated, not only by different test organisms, but also by variables in schedules of administering hormone and antigen (Dixon, 1954; Berglund, 1956) and amounts of cortisone and antigen given (Newsom and Darrack, 1956). The antiinflammatory effect of cortisone on host tissues has been observed for a variety of host-parasite combinations including clinical filariasis (March and Laigret, 1958), *Nematospiroides dubius* (Cross, 1956), *Nippostrongylus muris* (Weinstein, 1955), and most extensively for *Trichinella spiralis* (Coker, 1956; Markell and Lewis, 1957).

In comparing the role of antibody and phagocyte in cortisone-treated animals, Germuth (1956) has pointed out that cortisone functions to maintain capillary permeability in tissues exposed to inflammatory stimuli. The normal loss of blood proteins into such areas is thus reduced. Hence, the decrease in numbers of inflammatory cells in cortisone-treated tissues harboring nematode larvae may also be accompanied by decreased amounts of circulating antibody. The possibility of cortisone interfering with the functional ability of the macrophage or having a lytic effect on the various types of inflammatory cells appears unlikely (Germuth, 1956). The relative importance of inflammatory cells and antibody is further complicated by considering hypersensitivity to nematode infections. The presence of circulating

antibody as measured by serological techniques may not, therefore, be a valid measure of the resistance of a host to nematode larvae. Markell and Lewis (1957) have suggested a similar hypothesis for interpreting some of the findings in experimental trichinosis. Wharton (1947) has reported histopathological evidence from cotton rats of a hypersensitivity reaction to *L. carinii* worms. The marked increase in eosinophils in these infections also suggests that a hypersensitive state was induced by *L. carinii* larvae in both white and cotton rats.

SUMMARY

In experiments testing the resistance of white and cotton rats to the cotton rat filariid, *Litomosoides carinii,* it was found that immature white rats and cotton rats differed little in their resistance to a 10-day infection. Mature white rats developed an age resistance which was expressed by decreased percentages of larvae completing migration to the pleural cavities, and by increased encapsulation of larvae in the pleural cavities. The number of inflammatory cells in the pleural cavities of either species did not correlate with age or species differences in resistance. Daily cortisone injections (30 mg per kg) effectively suppressed the cellular response and the encapsulation of larvae in the pleural cavities of white rats, but did not increase the percentages of larvae completing migration. Immunization of white rats caused a decrease in the percentage recovery of challenging larvae, and an increase in the percentages of encapsulation. Immunization was most effective in mature rats as compared to immature rats. Cortisone was effective in suppressing the effects of immunization in white rats. The cellular response in the pleural cavities of both species of rats was markedly increased with infection, but did not differ quantitatively or qualitatively between white and cotton rats. A marked increase in the eosinophil count was noted in both hosts, while the remainder of inflammatory cells were primarily members of the monocytic series.

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DICYEMENNEA NOUVELI, A NEW MESOZOAN FROM CENTRAL CALIFORNIA*

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Smear preparations from two large octopuses taken in Monterey Bay, California (near Hopkins Marine Station, July 1947 by Dr. Nathan Riser; Pescadero Point, November 1947 by Dr. Donald P. Abbott), and two slides prepared by Dr. Riser from the squid *Loligo opalescens* Berry in September 1947 contain a new mesozoan here described.

Description: Dicyemennea; large, commonly reaching 2–5 mm length; total number of somatic cells 29 to 38 (most often 33 or 35); calotte orthotropal, swollen, usually somewhat pointed, the metapolar cells very large and contributing the chief element to the bulb-like swollen appearance of the calotte; parapolar cells similar to other trunk cells in character, not taking a prominent part in the cephalic swelling; trunk slender, of nearly uniform diameter throughout, without verruciform cells; uropolar cells somewhat shortened and swollen but not verruciform, the cytological aspect and granular content similar to that of the other trunk cells, most specimens terminating with a single unpaired uropolar cell; parapolars, regular trunk cells and uropolars charged with small irregularly shaped flattened granules of protein reserve in transitional and rhombogen individuals; nuclei of somatic cells spherical with prominent nucleoli and a very few small deeply staining chromatin granules on the inside of the nuclear membrane.

Vermiform larvae (figs. 4, 5, 6) elongate, attaining a length of about 150 to 175 microns before escape from the parent nematogen, the calotte already showing the characteristic swollen appearance in the fully developed ones; rudimentary second axial cell at anterior end of the axial cell; development of axial elements in enclosed vermiform larvae proceeding further than usual, with the production of 4 or more axoblasts before eclosion; vermiform larvae in individuals transitional between the nematogen and rhombogen phases also showing precocious nuclear phenomena such as production of paranuclei (fig. 4) or amitotic division of the axial nucleus (fig. 5).

Infusorigens (fig. 7) large, prolific; parent rhombogens often containing 3 or 4 infusorigens and dozens of infusoriform larvae in various stages of development.

Infusoriform (figs. 8, 9) 34 to 38 microns long, tapering rapidly to a blunt point behind, of typical appearance and structure for dicyemid infusoriforms.

Hosts: This species was found on slides from 2 large reddish octopuses taken in Monterey Bay, California, in July and November of 1947. It was also present on 2 slides from an otherwise negative series of preparations made from the kidneys of the squid, *Loligo opalescens* Berry, in September of the same year, also from Monterey Bay.

Types: Syntypes, slide 1108–6 (U.S.N.M. # 23607); paratypes on slides 1108, 1–24 (*Octopus*) and 1106, 1–2 (*Loligo*) in author's collection.

Discussion: Subsequent examination of 15 smaller octopuses and 16 squids from Monterey Bay by the author revealed only other species of dicyemids in the octopuses, and none at all in the squids. Over 200 octopuses and numerous squids from La Jolla, California, and other points ranging from Ensenada, Mexico, to Puget Sound, Washington, have also been examined without encountering this species.

The dicyemids from the octopuses appeared healthy and robust, mature nematogens, transitional individuals and rhombogens being present, with active generation of larvae taking place in their axial cells. Those on the 2 squid slides were very few in number, mostly nematogens, with less active reproduction taking place in their axial cells. Perhaps the octopus is the normal host for this species, while the infec-

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tion in the squid was accidental, the dicyemids not doing as well in the atypical host. However, since only 2 octopuses and, presumably, only 1 squid have been found infected with this species, definite conclusions about it cannot be made.

The octopuses were not preserved or definitely identified. The large reddish octopuses of the Pacific coast of North America north of Point Conception, California, have commonly been termed *O. apollyon* Berry or *O. hongkongensis* Hoyle. It is not entirely clear whether the large reddish octopuses of the eastern and western Pacific coasts are actually the same species or even whether those of the Pacific coast of North America constitute a single entity. Berry (1953) separated the smaller reddish ones from California as *Octopus rubescens* Berry.

The dicyemid fauna of the reddish octopuses of central California, so far as known (Nouvel, 1947; McConnaughey, 1941, 1949, 1957) differs so sharply from that found in octopuses from Puget Sound and the Oregon Coast as to suggest that different species of *Octopus* may be involved. In southern California a difference in dicyemid fauna provided one of the clues leading to the discovery that the *Octopus bimaculatus complex* actually comprises 2 species (Pickford and McConnaughey, 1949).

This species resembles *Dicyemennea eledones* Wagener but differs in possessing a greater more variable number of somatic cells in the vermiform phases (29–38, usually 33 or 35, as opposed to 23 in *D. eledones*). The infusoriform possesses 2 nuclei and 1 germ cell in each of the 4 cells in the urn, as do most dicyemids, instead of only 1 nucleus and 1 germ cell as in *D. eledones*. The infusoriform of *D. eledones* also has abnormal liquid filled vacuoles instead of the usual pair of large refractile bodies found in most dicyemids including the species here described.

The name *Dicyemennea nouveli* is proposed in honor of Dr. Henri Nouvel of Toulouse, France, who has done more than anyone else to put our understanding of the mesozoa in order.

ACKNOWLEDGMENTS

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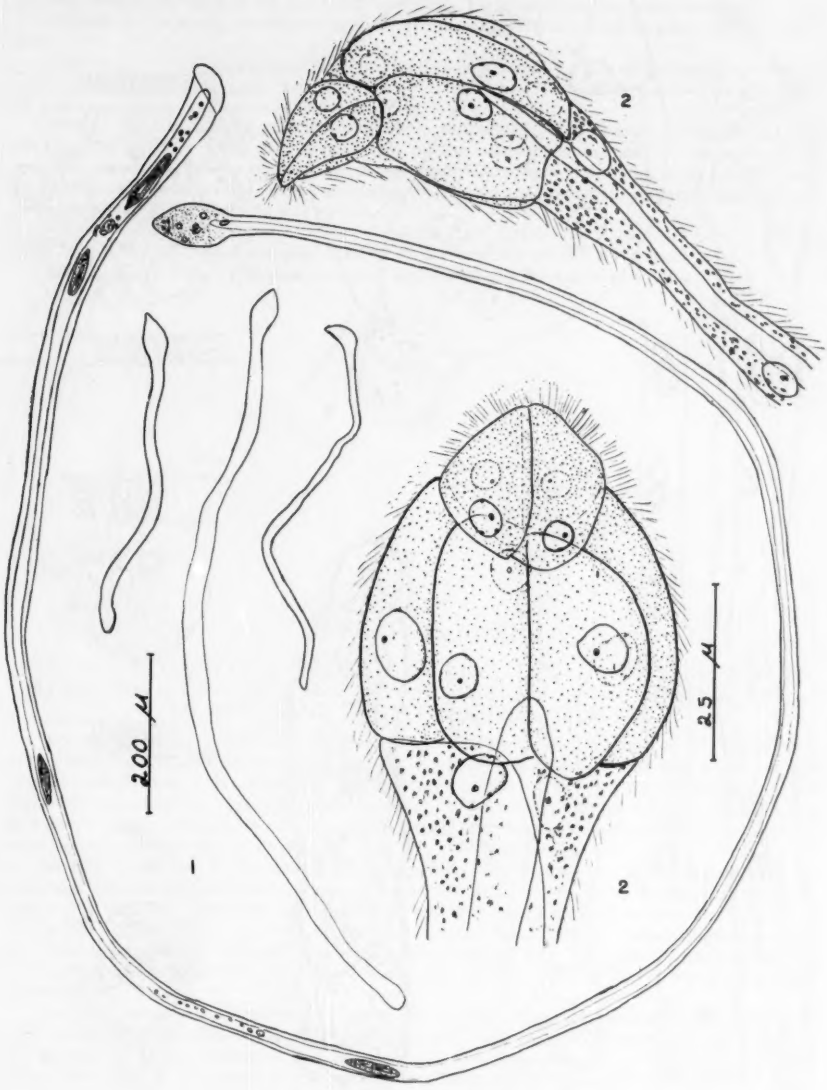
PLATE I



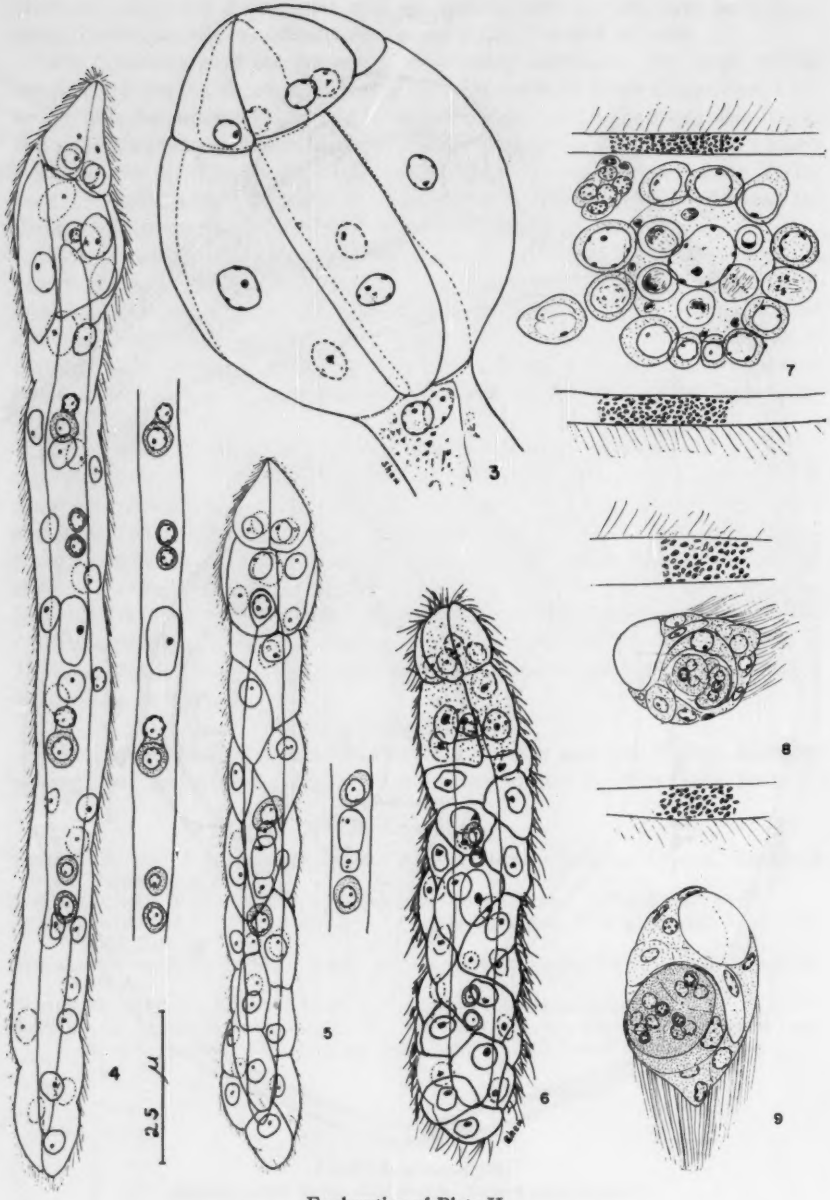
Explanation of Plate I

Dicyemennea nouveli; drawn with aid of camera lucida.

FIGURE 1. Outline drawings of 4 vermiform individuals to show size and proportions.

FIGURE 2. Calotte and anterior end of the trunk of 2 of the individuals shown in fig. 1.

PLATE II



Explanation of Plate II

Dicyemennea nouveli; drawn with aid of camera lucida.

FIGURE 3. Calotte of a large individual transitional between the nematogen and rombogen phases.

FIGURE 4. One of the vermiform larvae contained in the axial cell, showing 35 somatic cells, a small abortive second axial cell at the anterior end of the axial cell, early swelling of the calotte, and precocious elimination of paranuclei from 2 enlarged axoblasts.

FIGURE 5. A second vermiform larva in the same individual showing 32 somatic cells and amitotic unequal division of the axial nucleus giving rise to an accessory axial nucleus.

FIGURE 6. A young vermiform larva before swelling of the calotte has begun; 33 somatic cells.

FIGURE 7. Young infusorigen in optical section showing only a few of the many reproductive cells of which it is comprised. Orientation of chromatin at 1 pole of the nucleus prior to onset of meiotic divisions is shown in some of the cells.

FIGURE 8. Parasagittal, slightly oblique optical section of a mature infusoriform larva showing the contents of the urn, internal cilia on the anterior ventral wall of the cavity of the urn, the capsule cell of one side and the dorsal internal glycogen cell of the same side. Protein granules in the somatic cells of the parent rhombogen are also indicated, appearing flattened and somewhat irregular in shape in this species.

FIGURE 9. A similar view of a second infusoriform larva. All 4 germinal cells in the urn have been indicated though ordinarily only 2 or 3 are visible in any given focal plane.

(Figs. 1a, 1b, 4, 5, 7, 8, 9 from octopus; figs. 1c, 1d, 2a, 2b, 3, 6 from squid.)

RESEARCH NOTE

PRELIMINARY NOTE ON THE LARVAL DEVELOPMENT OF THE FRINGED TAPEWORM OF SHEEP, *THYSANOSOMA ACTINIOIDES* DIESING, 1834, IN PSOCIDS (PSOCOPTERA: CORRODENTIA).

The life history of the fringed tapeworm, *Thysanosoma actinioides* Diesing, 1834, a parasite of domestic and wild ruminants in the Americas, has remained unknown since this tapeworm was first recovered from a Brazilian deer by Natterer in 1824. This parasite probably occurred originally only in certain American wild ruminants, but now commonly parasitizes domestic sheep in western North America and wide areas of South America. The purpose of this note is to report recent progress on the elucidation of the life history of this parasite.

In 1957, Professor R. F. Honess of the University of Wyoming and the writer collected numerous arthropods from the habitats of moose, elk and bighorn sheep in the Jackson Hole region of Wyoming. They then fed these arthropods to domestic sheep in an unsuccessful attempt to trasmit *T. actinioides* to this host. There were among the arthropods collected a few psocids, insects which are known to occur naturally in areas frequented by range sheep. Since they had not been studied previously in the search for vectors of this parasite, they seemed to warrant investigation.

Two species of psocids were used in the experiments described herein, namely, *Liposcelis bostrychophilus* (fam. Liposcelidae; identified by Dr. Kathryn M. Sommerman), an apterous parthenogenetic species, and an undescribed psocid of the genus *Rhyopsocus* (fam. Psoquillidae; identified by Dr. A. B. Gurney), a winged bisexual species. Both species were easily cultured in the laboratory, and both readily ingested the egg capsules containing oncospheres of *T. actinioides*.

From 18 of 146 *L. bostrychophilus*, which were exposed to egg capsules of this tapeworm, a total of 54 larval cestodes were recovered. Although several weeks elapsed between exposure of some of the insects and their dissections, the most advanced larva encountered in this species was in the early blastula stage and measured only 67 by 54 microns. It remains to be determined whether development of the cestode can proceed further in this psocid.

Experiments with *Rhyopsocus* sp. were more successful. Five of 13 individuals of this species which were exposed to egg capsules of *T. actinioides* contained tapeworm larvae upon dissection. The diameters of the 13 larvae recovered from these five infected psocids ranged from 31 microns (about twice the diameter of the oncosphere and having an internal structure similar to that illustrated in A of fig. 1) to 105 microns, as shown in B of figure 1. This last mentioned larva was definitely a cysticercoid having a motile invaginated scolex. Two of the larvae were in the pre-invaginated stage with the cyst wall partially developed in the center of the elongated body. On the basis of the limited observations made to date, the larval development of *T. actinioides* in psocids resembles that reported for various species of anoplocephalid cestodes in oribatid mites. Characteristic differences, however, will probably be detected when more specimens become available for study.

Because all but a few of the psocids infected experimentally were reared in the laboratory, the possibility of their acquiring extraneous natural infections of the fringed tapeworm or other cestode species is extremely remote. Moreover, the dissimilarity between the embryonic hooks of *T. actinioides* and those of certain other tapeworms (Kates and McIntosh, 1950, J. Parasit. **36** (supp.) : 45) made is possible to identify positively some of the early larval stages recovered from these insects.

This is believed to be both the first report of the larval development of *T. actinioides* in any potential vector and the first report of the involvement of insects of the order Psocoptera (Corrodentia) in the life history of parasitic helminths.

Attempts to complete the life history of this cestode by administration of cysticercoids obtained from experimentally infected psocids to cestode-free lambs are now in progress.

The technical assistance of Mr. Kay Samson and Mrs. Patricia Jackson is gratefully acknowledged. This work was done in cooperation with the New Mexico Agricultural Experiment Station.—REX W. ALLEN, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, University Park, New Mexico.*

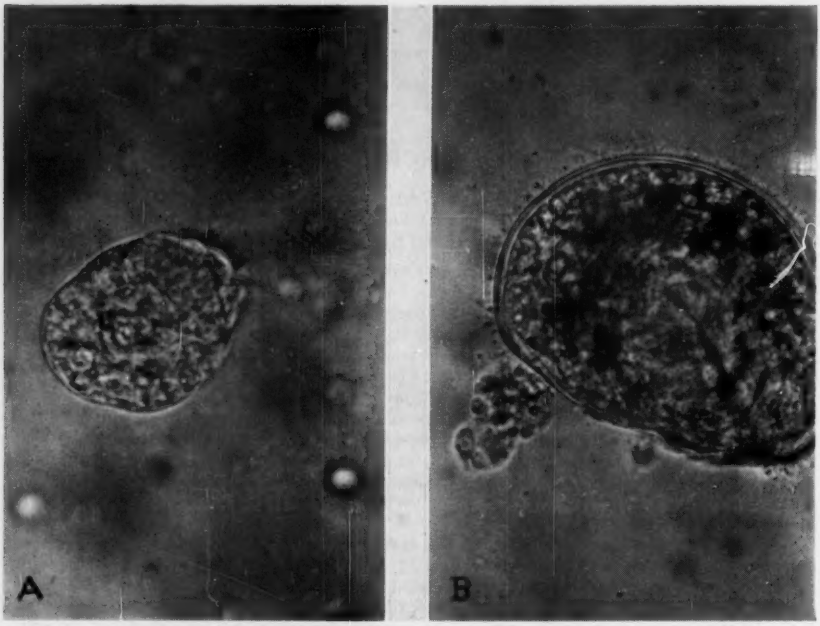


FIGURE 1. Larvae of *T. actinioides* recovered from experimentally infected *Rhyopsocus* sp. A, morula stage, diameter 53 microns; B, mature or nearly mature cysticercoid, 105 by 81 microns.

STUDIES ON THE GENUS *HOMALOMETRON* STAFFORD, 1904 (TREMATODA: LEPOCREADIIDAE), WITH A REDESCRIPTION OF *H. ARMATUM* (MacCALLUM, 1895)*

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During recent studies on parasites of Louisiana fresh-water fishes, a large series of trematodes belonging to the genus *Homalometron* was collected. This abundance of material and the confusion of similar species within the genus led the writer to undertake a thorough study of the genus. The original description (MacCallum, 1895) is old and brief, and in the light of present-day knowledge should be reviewed and supplemented.

The writer wishes to express sincere appreciation to Dr. H. J. Bennett of Louisiana State University for his constant aid and encouragement during these studies.

H. armatum is redescribed below and a list of synonyms is given. The hosts for this species in Louisiana are also given. A review of all other species assigned to this genus is presented.

OBSERVATIONS

Homalometron armatum (MacCallum, 1895) Manter, 1947 (fig. 2)

Syn: *Distomum isoporum* var. *armatum* MacC., 1895
Allocreadium armatum (MacC., 1895) Pearse, 1924
Anallocreadium armatum (MacC., 1895) Simer, 1929
Anallocreadium pearsei Hunter and Bangham, 1932

Hosts in Louisiana: *Aplodinotus grunniens, Lepomis humilis, Lepomis microlophus.*

Description: Observations based on 250 specimens and on measurements of 40. Average measurements in millimeters are shown in parentheses. Body flattened, elongate, 1.74–3.44 (2.66); maximum width 0.45–0.94 (0.71). Anterior two-thirds of cuticle spinate, entirely so in some cases. Acetabulum at juncture of 1st and middle thirds of body, highly muscular, with diameter of 0.18–0.51 (0.36). Oral sucker subterminal, round, with diameter of 0.15–0.35 (0.24). Prepharynx short, 0.04–0.19 (0.11); pharynx globular 0.06–0.17 (0.10) in length. Esophagus, very short, 0.03–0.09 (0.06), completely surrounded by glands. Intestinal bifurcation well anterior to acetabulum; ceca extending to posterior end of body.

Testes 2, tandem, intercecal, in posterior half of body; usually with a smooth surface, but sometimes slightly lobated. Anterior testis diameter 0.10–0.42 (0.30); posterior testis 0.14–0.47 (0.35). Vasa efferentia pass separately to level of acetabulum where they join to form a short vas deferens. The latter expands immediately to form large seminal vesicle which lies to one side of acetabulum. Genital pore in mid-line, immediately anterior to acetabulum.

Vitellaria with large follicles extending from level of acetabulum to posterior tip of body; confluent behind posterior testis, filling entire body in this area. Small vitelline reservoir immediately posterior to ovary.

Ovary midway between acetabulum and anterior testis; on right side of body; round to oval-shaped, 0.10–0.27 (0.17) in length, by 0.12–0.24 (0.19) in width. Very short oviduct immediately gives off conspicuous flask-shaped seminal receptacle and Laurer's canal. Ootype, not conspicuous, slightly dorsal and lateral to ovary. Uterus passes forward from ootype to genital pore as slightly coiled tube; confined to area between anterior testis and genital pore. Eggs, few in number, large, 0.071–0.115 (0.088) by 0.048–0.069 (0.058). Excretory bladder sac-shaped, opening at posterior tip of body.

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DISCUSSION

MacCallum (1895) described a distome from *Aplodinotus grunniens* (Raf.), the fresh-water drum, as *Distomum isoporum* var. *armatum*. Looss (1902) also observed this species and recognized the name given by MacCallum. In the opinion of Looss, this worm could not be included in the subfamily Allocreadiinae because it lacked a cirrus sac, but no taxonomic changes were made. Wallin (1909) also records this species as *Distomum isoporum* var. *armatum*. Pearse (1924), in his work on parasites of Wisconsin fishes, retained the name *armatum* but chose to include it in the genus *Allocreadium* Looss, 1900. Simer (1929) found it in *A. grunniens* from the lower Tallahatchie River in Mississippi. He cited the opinion of Looss (1902) regarding the subfamily, but did not designate a new subfamily. Simer erected a new genus and the form became *Anallocreadium armatum* (MacC. 1895) Simer, 1929. Hunter and Bangham (1932) described a new species in this genus as *Anallocreadium pearsei* from the same host, and erected a new subfamily, Anallocreadiinae, to contain the genus.

Manter (1936) remarked that the genus *Anallocreadium* showed affinities to the Lepocreadiinae through the genus *Homalometron*, but stated that further evidence of relationship should be sought in life history studies. Hopkins (1937) demonstrated, at least partially, the life cycle of *Anallocreadium armatum* and gave further evidence for familial relationships.

From the evidence presented by life history studies and from the apparent similarity of adult forms, Manter (1947) placed *Anallocreadium* Simer, 1929 in synonymy with *Homalometron* Stafford, 1904. He also named a new species *Homalometron elongatum* from *Gerres cinereus*, the grey mojarra, in Florida. Yamaguti (1953) agreed with Manter in this synonymy. (See table I.)

TABLE I. *Comparison of the features exhibited by species in the genus* Homalometron.

Features	*H. pallidum*	*H. armatum* Syn: *H. pearsei*	*H. elongatum*
Principal host	*Fundulus heteroclitus*	*Aplodinotus grunniens*	*Gerres cinereum*
Body length	2.72 mm	1.7–4.0 mm	1.9–3.1 mm
Body width	0.89 mm	0.45–1.5 mm	0.38–0.61 mm
Oral sucker	terminal?	subterminal	subterminal
Sucker ratio	1 : 1	1 : 1–1 : 2	1 : 0.9
Anterior extent of vitellaria	level of ovary	posterior edge of acetabulum	level of ovary
Egg size, microns	110 × 70	71–115 × 40–70	74–90 × 42–54
Glands	?	prominent esophageal glands	?
Major diagnostic characteristics	terminal oral sucker; sucker ratio 1 : 1; vitellaria mostly extracecal except behind testes	sucker ratio 1 : 1 to 1 : 2, vitellaria mostly extracecal, except behind testes	oral sucker with papillae; mouth elongate; sucker ratio 1 : 0.9; vitellaria between testes and ovary

The genotype of *Homalometron* is *H. pallidum* Stafford, 1904, from *Fundulus heteroclitis* at Woods Hole, Mass.; Linton (1901) described this form as *Distomum* sp. Subsequently, Stafford erected the new name without further description. In *H. pallidum* the vitellaria are both inter- and extra-cecal behind the posterior testis, but they are not shown as being confluent. In *H. armatum* the vitellaria have a similar distribution, but they are definitely confluent behind the posterior testis. Also, the spines on *H. pallidum* are described as being very small and easily overlooked,

whereas on *H. armatum* they are quite obvious. Therefore, *H. pallidum* and *H. armatum* are, apparently, separate species.

Hunter and Bangham (1932) described *H. pearsei* from 12 specimens taken from *A. grunniens*. They separated *H. pearsei* and *H. armatum* primarily on the relative size of the acetabulum in relation to other organs in the body. Using these characteristics, a number of specimens was selected at random from the present collection in order to establish their identity. An analysis of these studies has shown that *H. armatum* and *H. pearsei* cannot be separated. All variations occurred within the descriptions given for the 2 species. To confirm these observations, specimens were sent to Drs. Manter, Hunter, and Bangham. Hunter (personal communication) expressed the belief that the 2 species might be separated by applying statistics to overall measurements. This does not appear valid since the forms cannot be separated, even into 2 groups. Bangham (personal communication) stated that he could no longer be certain about separating the two into distinct species.

Manter (1947) described *H. elongatum* from the grey mojarra in Florida. His description would encompass the forms in this collection. More recently Bravo-Hollis & Manter (1957) show that the mouth of *H. elongatum* is elongate and its lateral edges bear 3 pairs of papillae. There is little doubt that *H. elongatum* is a valid species.

The esophageal glands of *H. armatum* are worthy of special consideration. Their presence is very noticeable in well-stained specimens, especially those specimens stained with Harris' haematoxylin. The writer has seen no reference to these structures in any of the forms assigned to the genus *Homalometron*. Perhaps they have not been mentioned because they were assumed to have little taxonomic significance. Their presence should be mentioned, however, in a morphological description of the worm.

SUMMARY

1. *Homalometron armatum* (MacCallum, 1895) Manter, 1947, is redescribed from 250 specimens collected from 3 host species (*Aplodinotus grunniens, Lepomis humilis,* and *L. microlophus*) from Louisiana.

2. *Homalometron pearsei* Hunter and Bangham, 1932, is shown to be synonymous with *H. armatum.*

3. A table of comparison of three valid species in the genus *Homalometron* is given.

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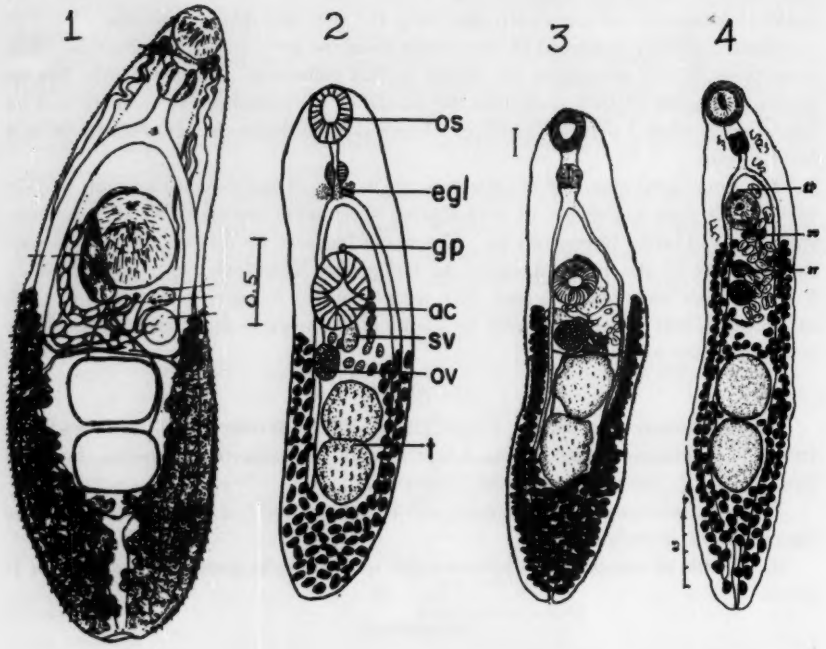
PLATE I



EXPLANATION OF FIGURES

(Figures 1, 2, and 3 have same scale; figure 4 has its own.)

FIGURE 1. *Homalometron armatum* (after MacCallum, 1895).

FIGURE 2. *H. armatum* (original): ac, acetabulum; egl, esophageal glands; gp, genital pore; os, oral sucker; ov, ovary; sv, seminal vesicle; t, testis.

FIGURE 3. *Homalometron pearsei* (after Hunter and Bangham, 1932).

FIGURE 4. *Homalometron elongatum* (after Manter, 1947).

STUDIES ON THE HOST PARASITE RELATIONSHIPS TO *SCHISTOSOMA JAPONICUM*. IV. RESISTANCE ACQUIRED BY INFECTION, BY VACCINATION AND BY THE INJECTION OF IMMUNE SERUM, IN MONKEYS, RABBITS AND MICE*

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Although not always substantiated by experimental evidence, the suggestion has been made by various authors that an initial schistosome infection limits the severity of subsequent infections (Fisher, 1934; Pesigan et al, 1958). Numerous reports on the development of acquired immunity with various forms of human and animal schistosomes have been reviewed by Khalil (1931), Bouillon (1950), Fairley (1951), Newsome (1956) and Kagan (1958).

With respect to the specific problem of acquired resistance to *S. japonicum*, Vogel and Minning (1953) reported that monkeys infected with male worms were still protected 1 to 3 years after treatment from otherwise lethal challenging doses. Lin and his coworkers (1954) also found that mice infected with male worms developed a moderate resistance to super infection. However, Hunter et al (1956) found no marked immunological response to reinfection in mice, hamsters and rabbits. They commented that these results are at variance with those obtained in the same laboratory 2 years earlier (Lin et al, 1954) and suggested that the selection of an experimental host for studies on immunological response to schistosomiasis may be a critical factor.

Attempts by various investigators to induce resistance by means of artificial immunization have been met with but little success and have often given conflicting results. Ozawa (1930), Kawamura (1932) and Lin et al (1954) reported that experimental animals were protected to a small degree by previous injections of saline suspension of whole *S. japonicum* worms. However, Vogel and Minning (1953) were unsuccessful in immunizing monkeys against infection with *S. japonicum* by this method.

Although diagnostic antibodies are present in the serum of infected animals, to demonstrate the presence of protective antibodies, the passive transfer of resistance should be successfully performed. Kawamura (1932) reported that he was able to protect dogs and rabbits by injecting them with serum from animals infected with *S. japonicum*. On the other hand, Vogel and Minning (1953) were unable to produce resistance in monkeys by injections with *S. japonicum* antiserum.

These conflicting results, often based on limited experimentation in different hosts, suggested that further work was needed on this problem, with more attention paid to the type of experimental animals, the size of immunizing doses, and the time interval between immunizing and challenging exposures. The experiments re-

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ported in this paper were carried out toward that end.

MATERIALS AND METHODS

Young adult white mice weighing approximately 20 g each, rabbits weighing approximately 2,000 g each, and Macaque monkeys (*Macaca irus*) about 2 to 4 years of age were used as test animals. Cercariae of *S. japonicum* were obtained by crushing infected snails collected from endemic areas. Not less than 10 infected snails were crushed at one time to assure the presence of both sexes among the infecting cercariae. The cercariae were counted and the animals were infected as described elsewhere (Sadun and Walton, 1958). Since some cercariae begin to lose motility a few hours after escaping from the snails, it was deemed important to infect only a limited number of animals at a time. Preliminary experiments indicated that the infectivity of cercariae varied with the age of the snails and the time of year at which the snails were collected. These observations are in agreement with those reported by Evans and Stirewalt (1951) who experimented with *S. mansoni*. Therefore, each experimental lot was separately controlled so that animals exposed to different lots of cercariae from snails collected at different times of the year could be tentatively compared.

Three antigens were used for the artificial immunization of mice and rabbits. A relatively crude, fat-free antigen was prepared from the adult worms, following essentially the technique described by Chaffee and his coworkers (1954). A metabolic antigen was obtained by incubating between 50 and 100 adult worms in a Carrel flask containing 5 ml of serum ultrafiltrate, 10 ml of Simm's X-6 solution, 15,000 units of penicillin and 15 mg of streptomycin. The mixture was shaken gently and incubated at 37° C for 72 hours, at which time the worms were removed from the preparation by centrifugation and the supernatant was lyophilized (Sadun et al, 1959). A cercarial antigen was prepared by grinding and extracting cercariae in saline. The cercariae were collected from the surface of water in which infected snails were placed. After protein determinations, these 3 antigens were diluted to a final concentration of approximately 100 mg of protein per 100 ml.

The antisera used for experiments on the passive transfer of resistance were obtained from rabbits. Some rabbits were infected with approximately 500 cercariae 8 to 12 weeks prior to the time they were bled. Others were divided into 2 groups: one receiving intramuscularly 2 injections of crude antigen adsorbed onto aluminum hydroxide (Dorin, 1946) 3 weeks apart, the other receiving metabolic antigen. The rabbits in these 3 groups were exsanguinated approximately 2 weeks after the second antigenic injection. These sera were positive by the complement fixation reaction against the homologous antigens, with titers varying between 1 : 32 and 1 : 128. A few rabbits were unexposed to cercariae and served as source of normal serum.

The number of worms recovered at necropsy in relation to the number of cercariae to which experimental and control animals were exposed, and the mean length of the worms, were used as criteria in determining resistance of the hosts to the schistosomes. The perfusion technique (Pan and Hunter, 1951) was used for recovery of the developing schistosomes. The recovered worms were sexed, counted, and fixed in Bouin's fluid. Where the length of the schistosomes was taken into consideration, tracings of the worms, magnified 5 times with a photographic enlarger, were measured with a map measure. Necropsies in mice were performed 30 days after exposure to the challenging infection. In monkeys and rabbits, necropsies were done 21–22 days and 25–26 days after the challenging infection, respectively. This interval allowed the schistosomula to reach the hepatic portal system and grow enough to be easily recovered. At this time, the worms from the challenging infection could be readily separated from those of the primary infection by their small size and incomplete development.

In the statistical analysis, the usual formulas for the values of t and P were used to determine the significance of the experimental results.

RESULTS

1. *Acquired Resistance.* Five series of mice were initially exposed to 30 cercariae each and challenged 10 to 80 days later with 80 cercariae each. As indicated in table I, an acquired immunity to super infection was present at 30 days and continued through the period of the experiment up to 80 days. The difference between the percent recovery of worms in the immunized animal in relation to the controls is significant at 30, 40 and 80 days, but not at 60 days. The data after 80 days are particularly significant, with a P value of less than 0.001. No appreciable difference in the sex ratio of developing schistosomes was noticed in the various groups. How-

ever, a larger number of stunted worms was observed in the immunized animals than in the controls.

TABLE I. *Number of* S. japonicum *recovered from mice challenged at various intervals after the primary infection.*

Experimental group	Number of cercariae per animal		Days between exposures	Results of examination at necropsy 30 days after challenging exposure			
	First exposure	Challenging exposure		Number of mice	Mean number of worms	Percent recovery	Probability
1	30	80	10	21	43	39	No reduc.
1C	0	80	—	19	29	36	
2	30	80	30	9	43	39	$P < 0.01$
2C	0	80	—	11	46	57	
3	30	80	40	11	34	31	$P < 0.01$
3C	0	80	—	12	40	50	
4	30	80	60	8	36	33	$P < 0.3$
4C	0	80	—	10	37	46	
5	30	80	80	10	24	22	$P < 0.001$
5C	0	80	—	11	50	62	

In the course of the experimental procedures in mice, the worms of the challenging infection were, at times, so advanced in their development that there was difficulty in distinguishing them from those of the primary infection. Therefore, no such separation was attempted. The results of this experiment also suggested that first exposure may bring about a reduction in the normal growth of the worms of the challenging exposure. In view of these considerations, in the following experiments, animals were necropsied between 3 and 4 weeks after the challenging exposure and measurements were made of the developing worms.

A second experiment was set up in rabbits to determine the effect that first infection would have on the number and size of worms that develop from a second infection. In this experiment, 14 rabbits were divided into 3 groups. Those of the first and second groups were exposed to 400 cercariae each, those of the third group were given no infection at this time. Seventy-five days after the first infection, all the rabbits of the first and third groups were given a challenging dose of 400 cercariae each. As indicated table II, the percent recovery of the challenging infection

TABLE II. *Number and length of* S. japonicum *recovered from rabbits challenged 75 days after a previous exposure.*

Experimental group	Number of rabbits	Number of cercariae per animal		Results of examination at necropsy 25–26 days after challenging exposure					
				First infection			Challenging exposure		
		First exposure	Challenging exposure	Mean number	Percent	Mean length in mm	Mean number	Percent	Mean length in mm
1	6	400	400	198**	49.5	8.0**	74*	18.5	4.1*
2	4	400	—	285**	71.2	8.8**	—	—	—
3	5	—	400	—	—	—	157*	39.2	5.7*

* $P < 0.001$. ** No significant difference.

was lower in the rabbits which had been previously infected than in the unexposed controls (groups 1 and 3). This difference is highly significant with a P value of less than 0.001. Similarly, the worms from the second exposure recovered from the immunized rabbits were shorter than those from the previously uninfected con-

trols. This difference is also highly significant. Regarding the primary infection, the 4 controls of group 2 harbored more and longer worms than the animals of group 1. These differences, however, are not significant.

A third experiment was set up along the same lines to test whether an acquired resistance is developed in monkeys as well as in mice and rabbits. Eight monkeys were divided into 3 groups. Those of groups 1 and 2 were exposed to 300 cercariae each and 70 days later all the monkeys of groups 1 and 3 were exposed to 400 cercariae each. As indicated in table III, fewer worms from the challenging expo-

TABLE III. *Number and length of* S. japonicum *recovered from monkeys challenged 70 days after a previous exposure.*

Experimental group	Number of monkeys	Number of cercariae per animal		Results of examination at necropsy 21–22 days after challenging exposure			
				First infection		Challenging exposure	
		First exposure	Challenging exposure	Number	Mean length in mm	Number	Mean length in mm
1	3	300	400	117	9.1**	39	2.31*
2	2	300	—	128	9.3**	—	—
3	3	—	400	—	—	185	3.93*

* $P < 0.001$. ** No significant difference.

TABLE IV. *Number of* S. japonicum *recovered from mice receiving antigen injections.*

Experimental group	Test solution	Results of examination at necropsy 30 days after exposure to 50 cercariae			
		Number of mice	Total number of worms	Percent recovery	Probability
1	saline	33	794	48.1	—
2	worm extract	34	666	39.2	$P < 0.05$
3	metabolites	40	761	38.0	$P < 0.001$
4	cercarial extract	7	149	42.5	Not significant

sure were recovered in the monkeys of group 1 than in those of group 3. A substantial difference was observed in the percent recovery of worms in the two groups. Similarly, the worms from the monkeys which had been previously immunized were smaller than those from the non-immunized controls (groups 1 and 3). This difference is highly significant with a P value of less than 0.001. No significant difference was found in the number and size of worms of the first infection in the animals of groups 1 and 2.

2. *Artificial Immunization.* An experiment was set up in the attempt to study the effect of repeated injections of antigens on the number of worms developing in experimentally infected mice. In this experiment, 114 mice were divided into 4 groups. Each mouse of group 2 received intraperitoneally 3 injections of 0.5 ml of adult *S. japonicum* antigen, 3 weeks, 2 weeks and 1 week before being exposed to infection. Each mouse of Groups 3 and 4 received a similar amount of metabolic and cercarial antigens, respectively. The mice of group 1 were given the same amount of saline and used as non-immunized controls. The results are summarized in table IV. At necropsy, the percent recovery of worms in the groups receiving antigens was lower than in the controls. The data resulting from experiments on mice receiving metabolites are particularly significant (P value less than 0.001).

3. *Passive Transfer of Resistance.* An experiment was set up in an attempt to

transfer resistance from rabbits which had been experimentally infected or artificially immunized, to young normal mice. A total of 109 mice were separated into 4 groups. Each animal of group 2 was given 2 ml of immune rabbit serum intraperitoneally divided into 2 equal doses, the first given 7 days and the second 3 days before exposure to the cercariae. Those of groups 3 and 4 received the same amount of serum from rabbits which were artificially immunized with crude adult and metabolic antigens. The mice of the first group received the same amount of serum from normal rabbits. The results summarized in table V show that at necropsy the mice in the 3 groups receiving antisera had an appreciably lower number of worms than those receiving normal rabbit serum. The reduction in the percent recovery of worms among the mice receiving metabolic antisera was not statistically significant.

TABLE V. *Number of* S. japonicum *recovered from mice receiving antiserum injections.*

Experimental group	Type of serum	Results of examination at necropsy 30 days after exposure to 50 cercariae			
		Number of mice	Total number of worms	Percent recovery	Probability
1	Normal	30	634	42.2	—
2	Immune	29	475	32.8	$P < 0.05$
3	Worm extract antiserum	26	428	32.9	$P < 0.05$
4	Metabolites antiserum	24	424	35.3	Not significant

SUMMARY AND CONCLUSIONS

An analysis of the data of the foregoing experiments reveals, in general, that monkeys, rabbits and mice develop an acquired resistance to *Schistosoma japonicum* following previous infection with the same trematode. This resistance is manifested by the reduction in number and size of the developing worms. No difference in the sex ratio of developing worms was observed in the immunized animals.

Attempts to develop resistance in mice, both with worm extracts and worm metabolites, were also successful. Likewise, a certain degree of resistance to the development and growth of the parasites was conferred by injections of immune sera. The resistance conferred by injections of antigens and by passive transfer of serum was less marked than that encountered in animals which were immunized by a previous experimental infection. This was to be anticipated since, in animals immunized by active infection, there is a continuous production of antibodies as long as the antigenic stimulus persists. Our results are at variance with those of Vogel and Minning (1949a, 1949b) who failed to observe resistance in animals which had received worm extracts and sera from infected animals.

The results presented here add support to the evidence already available that animals develop an acquired immunity to *S. japonicum* and that this immunity is partly, at least, serological in nature. Studies are being currently carried out to determine the relative role of a cellular and humoral response in the defense mechanism to *S. japonicum* infection.

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RESEARCH NOTE

OCCURRENCE OF THE LUNG FLUKE *PARAGONIMUS RUDIS* (DIESING, 1850) IN NATIVE PIGS IN GEORGIA.

Insofar as the writers are aware, there are no previous authentic records of *Paragonimus* from native domestic animals in Georgia. Cooperrider (1952, Vet. Med. **47:** 65–70) recorded Paragonimus from pigs slaughtered at a packing plant in Atlanta, Georgia, but the origin of these pigs is not certain. McKeever (1958, J. Parasit. **44:** 324–327) reported 53 autochthonous *Paragonimus* infections of 4 genera of Georgia mammals. Jordan and Byrd (1958, J. Parasit. **44:** 470) also recorded its occurrence in an additional host from Georgia.

In April 1954 the lungs of a pig submitted to the diagnostic laboratory at Tifton, Georgia, were referred to the writers for parasitological examination. According to the owner, the animal, which was a typical "piney woods rooter," had been farrowed and raised on a farm near Fitzgerald, Georgia. Grossly, the lungs showed several dark-red nodules, some of which measured as much as 2 cm in diameter. These nodules extended deep into the lung tissue, and when incised were found to contain a viscous reddish-brown fluid and 2 or 3 small flukes which were tentatively identified as *Paragonimus*. Some of these specimens were sent to Dr. Allen McIntosh at the Beltsville Parasitological Laboratory, Beltsville, Maryland, who identified them as immature *Paragonimus rudis* (=*Paragonimus kellicotti*). These were deposited in the U. S. National Museum Helminthological Collection as No. 49428.—T. B. STEWART AND D. J. JONES, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Tifton, Georgia.*

STUDIES ON THE HOST PARASITE RELATIONSHIPS TO *SCHISTOSOMA JAPONICUM*. V. REACTIONS IN THE SKIN, LUNGS AND LIVER OF NORMAL AND IMMUNE ANIMALS FOLLOWING INFECTION WITH *SCHISTOSOMA JAPONICUM**

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Monkeys, rabbits and mice infected with *S. japonicum* develop an increased resistance to subsequent infections (Vogel and Minning, 1953; Lin et al, 1954; Sadun et al, 1959).

Very little information is available as to the mechanisms involved in this resistance, although it is assumed that inflammation of the skin, lungs and liver, which lie along the path of migration of the worms, must lead to the destruction of the parasites. Lewert and Lee (1954) have shown that a thickening of the skin, especially the basement membrane in all animals, holds back a large proportion of cercariae. This thickening occurs with age, after hypophysectomy, and under other experimental conditions. Stirewalt's observations (1953) that an area of mouse skin exposed to cercariae was resistant for approximately 2 days to subsequent infection, would indicate a local immune reaction. Vogel and Minning (1953) reported a high mortality of schistosomula when they passed through the lungs of immune animals. Since the young worms must reach the portal system before their penetration glands atrophy in the course of development, any delaying action on the part of the host would considerably reduce their chances of reaching the portal veins and result in an increased resistance to infection (Newsome, 1956).

The presence of a humoral mechanism of defense has been suggested by various investigators. Injections of some of the developmental stages of *S. japonicum* have produced in experimental animals a moderate degree of protection to a challenging infection (Ozawa, 1930; Kawamura, 1932; Lin et al, 1954; Sadun and Lin, 1959). Investigations by Kawamura (1932) and by Sadun and Lin (1959) indicated the presence of protective antibodies by transferring resistance from experimentally infected and artificially immunized animals to susceptible animals.

It was believed that studies on the mechanism of resistance to *S. japonicum* require investigations on the delay of schistosomula by means of cellular and/or serological reactions in the skin, lungs and liver of resistant animals. Therefore, the following experiments were designed primarily to compare the cellular response to cercariae of *S. japonicum* in normal and immune animals and to determine the relative role played by the inflammatory response in the skin, the lungs and the liver, of immune animals as areas of defense against infection. Detailed comparisons of the cellular reactions were made in initially infected animals and in others after various degrees of immunization produced by reinfection.

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MATERIALS AND METHODS

This investigation was carried out on young adult white mice, white rabbits and monkeys (*Macaca irus*). The animals were exposed to *S. japonicum* cercariae as described previously (Sadun et al, 1958; Sadun and Walton, 1958). Small circles were marked on the shaved skin with india ink at the time of exposure, and cercariae were placed within these circles with a hair loop. At necropsy, the mice were opened by a ventral incision and the entire animal was placed rapidly in a jar containing 10 percent formalin. Strips of skin obtained from the ventral surface of the abdomen of the rabbits were pinned on cork before fixation to facilitate sectioning. The skin, and parts of the lungs and liver were embedded in paraffin. Sections were cut at 8 microns and stained with dilute Delafield's hematoxylin and counterstained with eosin.

The skin, lungs and livers from 3 groups of 30 mice each were examined. One group was designed to determine the normal course of initial infection. Each mouse was exposed to 100 cercariae and 2 mice were killed at each of the following intervals: 1, 4, 8, 16, 20, and 24 hours, and 2, 3, 4, 5, 8, 10, 14, 21, and 28 days after infection. Another group was designed to determine the course of infection in recently immunized animals. Each mouse was exposed to 25 cercariae 3 times, at 3-day intervals, and challenged with 100 cercariae, 2 weeks after the last immunizing dose. Two mice were sacrificed at each of the time intervals listed above. A 3rd group was designed to study the development of immunity in old infections. Each mouse in this group was exposed to 5 cercariae 3 times at 3-day intervals and challenged 9 weeks after the last immunizing dose. Two mice were killed at each of the time intervals listed before. The tissues from a few normal mice of similar ages kept under similar conditions were examined as controls for these experiments, to make certain that no infections or other cause was producing inflammatory changes which might obscure the experimental results.

The skin, lungs and livers from 2 groups of rabbits were examined. One group consisted of 4 rabbits initially infected with 400 cercariae and was designed to determine the normal course of initial infection. Tissues were obtained from the rabbits at each of the following intervals: 1, 4, 8, and 24 hours, and 2, 3, 5, and 15 days after infection. A 2nd group consisted of 5 rabbits initially infected with 500 cercariae and re-exposed 9 weeks later to determine the course of infection in immunized animals. Tissues were obtained at the same time intervals as before.

The lungs and livers from 8 monkeys were examined. One group consisted of 3 monkeys initially infected with 600 cercariae and was designed to determine the course of initial infection. A 2nd group consisted of 5 monkeys initially infected with 300 cercariae and challenged 14 weeks later, to determine the course of infection in immunized animals. All these animals were sacrificed 19 and 21 days after the challenging infection and sections of liver and lungs were prepared.

EXPERIMENTAL RESULTS

Findings in mice following an initial infection. One hour after exposure, schistosomula were found in the epidermis and dermis. Some appeared in the hair follicles. Four hours after exposure, a few were still in the epidermis but most had reached the dermis and subcutaneous tissue (fig. 1). A very small number of heterophil cells was observed in the tunnels in the epidermis where the cercariae had penetrated. Sixteen hours after exposure, a few schistosomula were still in the epidermis, although most were in the dermis. Some diffusely scattered eosinophils and pseudo-eosinophils could be seen in the dermis (fig. 2). Forty-eight hours after exposure, the schistosomula were present in the dermis still without any noticeable cellular response around them. There were some mononuclear cells and a few pseudo-eosinophils present. Only 1 schistosomula was observed in the dermis 3 days after exposure. All schistosomula had left the skin by the 4th day after exposure, and a day later the skin appeared completely normal. The cellular response of the skin of mice during initial infection was very mild. Few scattered pseudo-eosinophils and occasional eosinophils were present in the dermis.

The young schistosomula were first observed in the lungs 16 hours after exposure and were without any apparent cellular response. After 6 days, the schistosomula could no longer be found in the lungs, although hemorrhages were observed up to 10 days after exposure. Twenty-four hours after infection, there was some slight perivascular infiltration consisting of pseudo-eosinophils, mononuclear cells and

occasional eosinophils. A mononuclear type of cell infiltration was observed up to 10 days after infection and was limited to the small blood vessel in the lungs.

Schistosomula were first found in the liver 3 days after exposure. Their number increased gradually up to the end of the experiment, 28 days after exposure. Four days after infection, a slight accumulation of pseudo-eosinophils, eosinophils and mononuclear cells was observed around the periportal areas. This accumulation of cells increased up to 8 days after infection and reverted to the mononuclear type prior to the deposition of eggs. Twenty-eight days after infection, eggs and worms were observed in the liver parenchyma and portal veins, respectively, without any noticeable cellular response.

Findings in mice challenged two weeks after immunizing exposures. The sequence of events immediately after the challenging exposure to cercariae was similar to that described for the mice of the first group. However, there was a slight cellular response which appeared a little earlier. Mononuclear cells were observed 24 hours after exposure, and reached the greatest number 5 days after exposure. No schistosomula were retained in the skin on the 4th day and thereafter.

Schistosomula were present in the lungs 24 hours after exposure, and were no longer found on the 5th day and thereafter. The lungs of 1 mouse revealed localized pneumonitis 48 hours after exposure. Hemorrhages were observed at all times from 24 hours to 28 days after exposure. A slight accumulation of mononuclear cells was observed around the small blood vessels of the lungs. Some eggs from the immunizing infections were seen in the blood vessels 10 and 14 days after the challenging infection without any noticeable cellular reaction.

Three days after the challenging exposure, corresponding to about 3 weeks after the immunizing exposures, worms and eggs were observed in the portal veins and in the liver parenchyma without any cellular response around them. Four days after the challenging exposures, there were small abscesses consisting primarily of pseudo-eosinophils with some lymphocytes and occasional eosinophils. Coagulative hyaline necrosis of parenchymal cells was first observed 8 days after the challenging exposure. The abscess formation and necrosis had become massive and widespread 10 days after challenging exposure. Schistosomula were observed in the liver up to the end of the experiment.

Findings in mice challenged nine weeks after immunizing exposures. The cellular response in the animals of this group was strikingly greater and occurred at an earlier time than in the previous group. Cells surrounded the cercariae as early as 4 hours after exposure (fig. 3). Sixteen hours after exposure, there was already a dense accumulation, primarily pseudo-eosinophils, and abscess formation along the tunnels (fig. 4) in the epidermis where cercariae had penetrated. The inflammatory cellular response increased gradually with time (fig. 5) and reached the peak 3 days after the challenging exposure. Four days after exposure, no schistosomula were seen in the skin. The acceleration and accumulation of cellular response around the schistosomula were the most distinguishing characteristics of this group of mice. However, there was no evidence that the schistosomula in the skin were invaded by the wandering cells.

The schistosomula were found in the lungs 3 days after the challenging exposure and persisted up to the end of the experiment 28 days after infection. The mononuclear cell infiltration around the small blood vessel was observed as early as 16 hours, and persisted until the end of the experiment. Hemorrhages of the lungs

were noticed beginning with the 2d day. Partial acute bronchiolar pneumonitis was also observed between 20 hours and 4 days after the challenging infection. Fourteen days after infection, abscesses surrounding eggs and disintegrated schistosomula were found. At this time, a large number of underdeveloped schistosomula were found in the lungs either in the arterioles or, occasionally, in dilated alveolar cavities (fig. 6). Twenty-one days after infection, 1 disintegrated schistosomulum was found in the alveolar cavities and was partially invaded by cell infiltration (fig. 7). Twenty-eight days after infection, schistosomula in the blood vessels were surrounded by mononuclear cells (fig. 8). Some worms were in the process of disintegration with thrombosis of the vessel and intense perivascular accumulation of pseudo-eosinophils and mononuclear cells. The vessels were partly or completely surrounded by wandering cells, but, in some instances, this infiltration could not be distinguished from that taking place where worms were absent. Many schistosomula appeared to be stunted, blocked in blood vessels, and surrounded by accumulation of inflammatory cells. Eventually, many died and became infiltrated by phagocytic cells.

In the liver parenchyma, eggs were found surrounded by inflammatory cells, primarily pseudo-eosinophils with some lymphocytes and occasional eosinophils. In addition, numerous pseudo-tubercles and small areas of hyaline necrosis were noted. As time progressed, the inflammatory processes developed to large lesions, and necrosis became massive and the abscess formations became widely spread. The abscesses were surrounded by fibrous tissue 28 days after infection. The schistosomula were not encountered in the liver until the 8th day after the challenging infection, except in one animal where a few were seen after the 3rd day. The schistosomula appeared to be stunted and the reproductive system was much less developed than that of the worms of the same age found in the non-immune mice.

Findings in rabbits following an initial infection. One hour after exposure, most of the schistosomula were found in the epidermis, hair follicles and superficial parts of the dermis without any noticeable cellular response. A few pseudo-eosinophilic leukocytes were seen in the tissues where the schistosomula passed through 4, 8, and 24 hours after exposure (figs. 9, 10). Twenty-four hours after exposure, the capillaries in the dermis were congested, and some of them showed a pseudo-eosinophilic cell infiltration. Mononuclear cells were observed. Forty-eight hours after exposure, most of the schistosomula were present in the dermis without noticeable cellular response. The capillaries in the dermis at this time were still somewhat congested. Five days after exposure, the skin had almost reverted to normal.

At this time, schistosomula were found in the center of hemorrhagic areas in the lungs almost without cellular reaction. One schistosomula was observed in the portal vein in the liver, also without cellular reaction. Fifteen days after exposure, the skin, the lungs and the liver had reverted to their normal histological picture.

Findings in rabbits challenged nine weeks after an immunizing exposure. One hour after exposure to the challenging infection, the schistosomula were found in the epidermis, dermis and hair follicles. Four hours after infection, the schistosomula were already surrounded by a heavy accumulation of neutrophiles (fig. 11), and the tunnels where the cercariae penetrated in the epidermis revealed a dense accumulation of pseudo-eosinophils and abscess formation. The capillaries in the dermis were congested and infiltrated with pseudo-eosinophils. The schistosomula appeared at times degenerated with piknosis and fragmentation of the nuclei. Eight

hours after infection, abscess formations were observed in the epidermis where the schistosomula were present (fig. 12). The capillaries were congested and filled with pseudo-eosinophils. One schistosomula had degenerated in the epidermis and was surrounded by cells. Essentially the same picture was observed 72 hours after infection, after which time no schistosomula were found in the skin. Fifteen days after infection, the skin appeared essentially normal.

In the lungs, some hemorrhagic areas with schistosomula in the center were observed 72 hours after infection. Some eggs were seen in the alveolar walls without cellular response. Others were surrounded by a heavy accumulation of mononuclear cells. Fifteen days after infection, numerous abscesses and pseudo-tubercles surrounding the eggs were observed. Acute abscesses around the eggs with localized pneumonitis and often central necrosis and giant cell formation were observed.

In the liver, there were many lesions with the egg surrounded by a wide zone of necrotic cells showing nuclear fragmentation. This zone, in turn, was surrounded by epithelioid cells, some of which were surrounded by fibrous pseudo-tubercles in the portal spaces. In the liver, pseudo-tubercles were also seen surrounded by fibrous tissues. The histological picture of the immunized rabbits contrasted greatly with that of the animals during the initial infection. A peripheral zone of dense fibrous tissue sharply separated from the surrounding normal liver parenchyma was observed. Some lesions had advanced entirely to the stage of fibrosis. There was a great increase of fibrous tissue in the portal spaces, extending between them and forming a definite pattern of cirrhosis out of all proportion to the number of eggs present.

Findings in monkeys following an initial exposure. Essentially normal lungs and almost normal livers were observed in the monkeys about 3 weeks after their initial exposure. Schistosomula were found in the portal veins of the liver without any cellular response around them. At this time, a slight mononuclear cell accumulation was observed in the blood vessels in some parts of the lungs. Nearly 4 months after exposure to the initial infection, some pseudo-tubercles were observed in the lungs. The eggs were surrounded by central necrosis of tissue, and giant cell formation of eosinophils and epithelioid cells was seen. Some parts of the bronchioles showed bronchiolitis. Most of the lung tissue, however, appeared normal. No perivascular cell infiltration was observed at this time. No coagulative necrosis of the parenchyma cells was observed. Some of the eggs were surrounded by giant cells, epithelioid cells, eosinophils, mononuclear cells and fibroblasts. Hematin pigment was present in mononuclear phagocytes in the portal spaces and in the Kupffer cells. The liver parenchyma appeared essentially normal. Most of the eggs found in the liver were normal, containing viable miracidia, but some of them were merely empty shells and a few were calcified. Generally, the cellular infiltration was limited to the periportal spaces and did not extend into the parenchyma. The pseudo-tubercles were more fibrous than those observed in mice and rabbits. The liver lesions did not appear to be as massive as in mice and rabbits.

Findings in monkeys challenged fourteen weeks after an immunizing exposure. More pseudo-tubercles were observed in the lungs of animals of this group. Some egg lesions were present in the stage of acute abscesses with many eosinophils, some in the stage of pseudo-tubercle formation with giant cells, and some surrounded by fibrous tissue. Mononuclear cells were accumulated around the blood vessels. Also, in the liver, more pseudo-tubercles were observed with a massive accumula-

tion along the periportal spaces extending out into the liver parenchyma. However, no coagulation necrosis of the liver cells was observed. Hematin pigment was found in the Kupffer cells.

DISCUSSION

In earlier experiments (Sadun and Lin, 1959), it was demonstrated that a previous infection with *S. japonicum* rendered animals resistant to subsequent infections. Also, a certain degree of resistance to the development and growth of the parasites was conferred by injections of immune sera. This indicated that the acquired immunity is partly, at least, serological in nature.

The present series of experiments was carried out primarily to gain additional information on the relative roles of cellular and humoral responses in the defense mechanism to *S. japonicum* infection in immune animals. Miyagawa (1913) did not find any obvious cellular reaction surrounding cercariae of *S. japonicum* in the skin of experimental animals. Narabayashi (1916) and Sueyasu (1920) noticed hemorrhages and cellular infiltration around the cercariae in the skin of the infected mice. Faust and Meleney (1924) reported a definite tissue reaction around cercariae in the skin of a dog 15 hours after exposure, but were unable to observe any tissue reaction in the skin of a mouse 24 hours after exposure. Miyagawa and Takemoto (1921) observed no cutaneous reactions to the penetration of the cercariae in mice 20 to 28 hours after infection, but very intense inflammation of the lungs. Likewise, according to Kagan and Meranze (1955), Takemoto saw no cellular reaction in the skin of forearm 2 hours after penetration of cercariae. In a comparison of the cellular responses in the skin of normal and immune rats, Watarai (1936) reported that the response varied with the number of cercariae used. He also observed that in hyperimmune animals, the inflammatory cells appeared to be more aggressive in attacking the cercariae.

The results of our series of experiments, in general, indicate that the cellular reactions in the tissues of immunized animals occurred earlier and were much more marked than in the non-immune controls. The difference between normal and immune animals appeared to be quantitative in the sense of tissue allergic reaction and was much more evident when the challenging infection occurred at least 2 months after the immunizing infections. The cellular reaction in the skin of immunized animals was such as to indicate that the migrations of the schistosomula were slowed down. In fact, whereas in normal mice the schistomula reached the lungs in 16 hours after infection, in recently immunized mice, they reached the lungs 24 hours after infection, and in mice which had been challenged over 2 months after the immunizing exposures, they reached the lungs 2 days after infection. In this group, a large number of schistosomula were trapped in the lungs where they were still found at the end of the experimental period 28 days after the challenging infection. The results in mice, rabbits, and monkeys were essentially similar.

Bronchiolar pneumonitis was observed frequently in the immunized animals at the time the schistomula of the challenging infection passed through the lungs. This phenomenon could be interpreted as being due to hypersensitivity resulting from the secretory products of the schistosomula. This suggestion is supported by the observation that pneumonitis was found frequently in the mice challenged 9 weeks after the immunizing infections, occasionally in those challenged 2 weeks after the immunizing infections, and never in those which had not been previously immunized.

When the worms were immobilized in the skin or the lungs, inflammatory reactions appeared around them and nodules were formed. In many instances, the schistosomula were killed and the macrophages and giant cells in the nodules appeared to be in the process of removing the debris. Those worms which apparently were able to escape the barrier of the skin and lungs were seen in the liver of the immune animals, obviously stunted and underdeveloped.

Whether the cells themselves were able to slow down and stop the schistosomula, or whether there was an antibody action against the worms, preventing normal migration and growth, could not be determined with certainty, since no visible precipitates around the worms in the tissues were seen. If the inhibitory activity on the development of the parasites could be duplicated in passively immunized animals, the exact role of antibodies could probably be better evaluated. However, the similarity of our results to those reported by Taliaferro and Sarles with *Nippostrongylus muris* (1939) is such as to suggest that in *S. japonicum* also the mechanism of immunity operates both by humoral and cellular means. This conclusion appears all the more warranted when considered in conjunction with our earlier studies (Sadun and Lin, 1959).

SUMMARY

The cellular changes in the skin, lungs, and liver of normal and immune animals after infection with *Schistosoma japonicum* were studied.

The results in monkeys, rabbits, and mice were essentially similar. A very mild, diffuse cellular reaction was observed in initially infected animals. Conversely, in animals which had been infected at least 2 months previously, an intense cellular reaction occurred a few hours after penetration of the cercariae, and the migration of the schistosomula was slowed down. Some schistosomula were trapped in the skin, others were able to reach the lungs where most of them seemed to be blocked and surrounded by diffuse nodules where they remained alive for long periods. Among those which succeeded in escaping, several were seen in the liver, where they appeared stunted and sexually underdeveloped. Several worms were observed dead and infiltrated with phagocytes.

On the basis of these observations and those of our previous studies, the conclusion was reached that both cellular and humoral responses play an important role in the defense mechanism of immune animals to *S. japonicum* infection.

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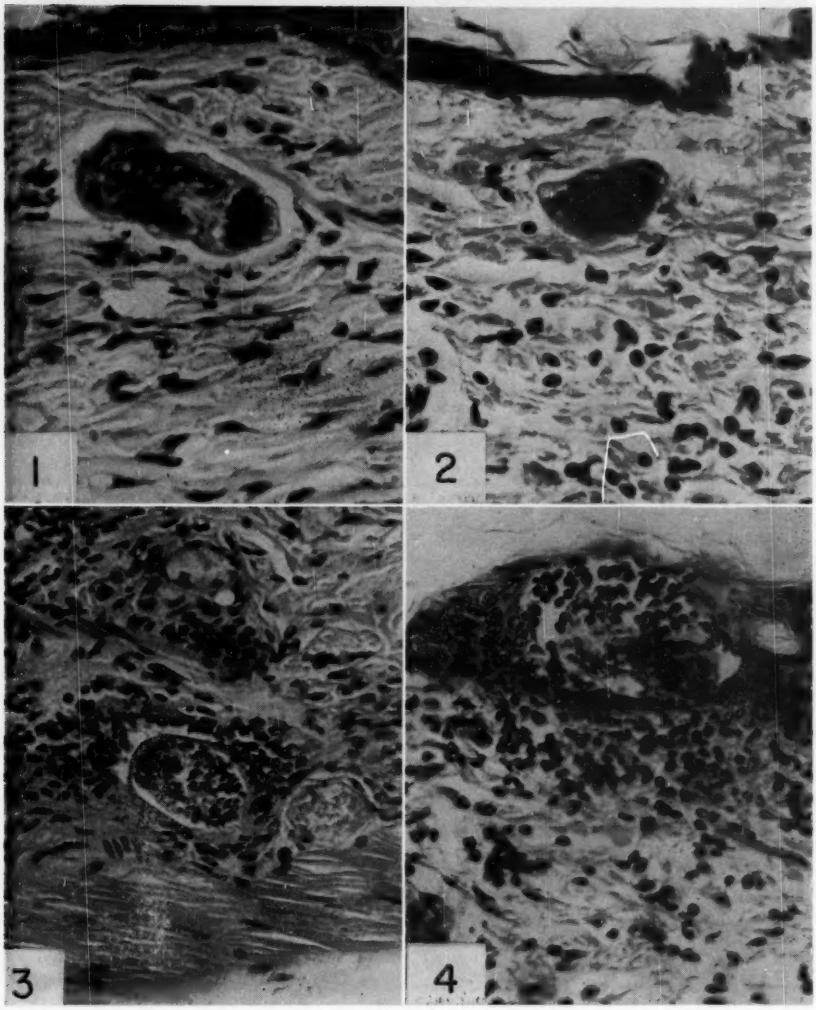
PLATE I



EXPLANATION OF PLATES

FIGURE 1. Initial infection in mice. Schistosomula in the dermis, without cellular reaction, 4 hours after infection.

FIGURE 2. Initial infection in mice. Schistosomula in the dermis with a slight diffusely scattered cellular reaction, 16 hours after infection.

FIGURE 3. Immune mice. Schistosomula in the subcutaneous tissue surrounded by dense accumulations in inflammatory cellular reaction, 4 hours after infection.

FIGURE 4. Immune mice. Schistosomula in the epidermis surrounded by leukocytes and early abscess formation, 16 hours after infection.

PLATE II

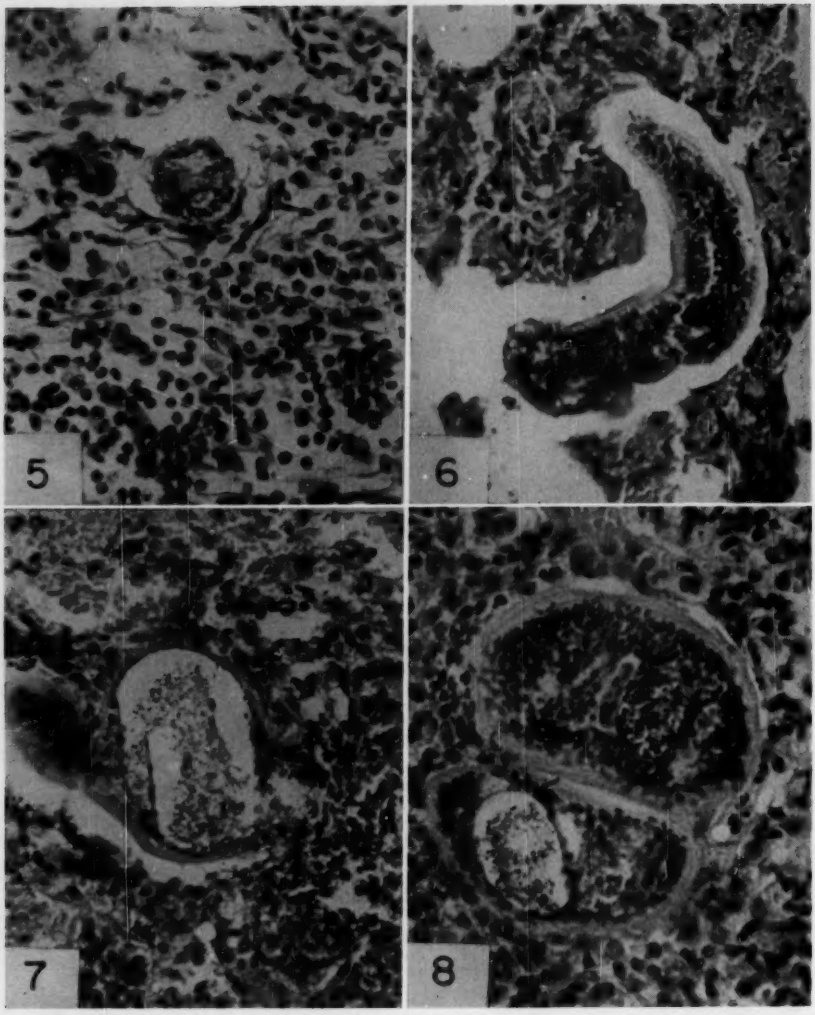


FIGURE 5. Immune mice. Schistosomula in the dermis surrounded by heavy accumulation of inflammatory cells, primarily pseudo-eosinophils with some eosinophils, lymphocytes and histiocytes, 24 hours after infection.

FIGURE 6. Immune mice. Schistosomula present in the dilated alveolar cavities, surrounded by hemorrhages, with pseudo-eosinophils and mononuclear cell infiltrations, 14 days after infection.

FIGURE 7. Immune mice. Disintegrated schistosomula in the alveolar cavity of the lung, surrounded by hemorrhages and accumulation of mononuclear and phagocytic cells, 21 days after infection.

FIGURE 8. Immune mice. Schistosomula surrounded by accumulation of mononuclear cells in the alveolar cavity of the lung, 28 days after infection.

PLATE III

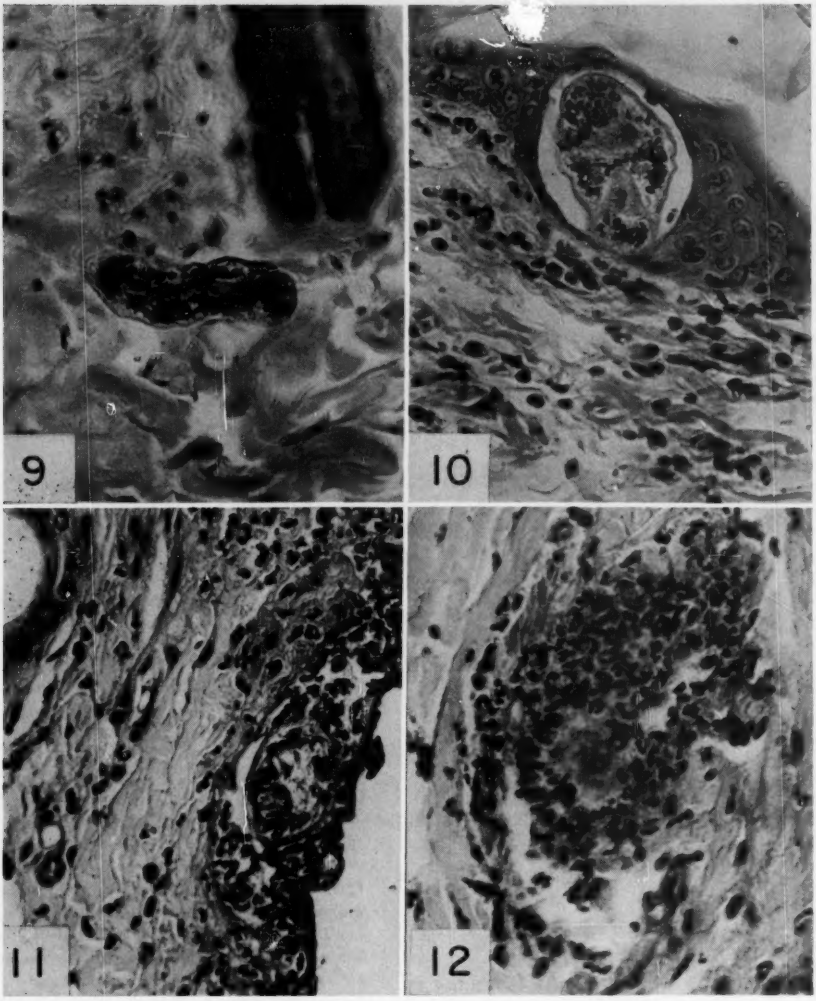


FIGURE 9. Initial infection in rabbits. Schistosomula in the dermis without cellular reaction, 4 hours after infection.

FIGURE 10. Initial infection in rabbits. Schistosomula in the epidermis without cellular reaction. Some pseudo-eosinophilic and eosinophilic leukocytic infiltration scattered diffusely in the dermis, 8 hours after infection.

FIGURE 11. Immune rabbits. Schistosomula in the epidermis surrounded by heavy accumulation of pseudo-eosinophils and occasional eosinophils, 4 hours after infection.

FIGURE 12. Immune rabbits. Schistosomula in the dermis surrounded by heavy accumulation of pseudo-eosinophils, 8 hours after infection.

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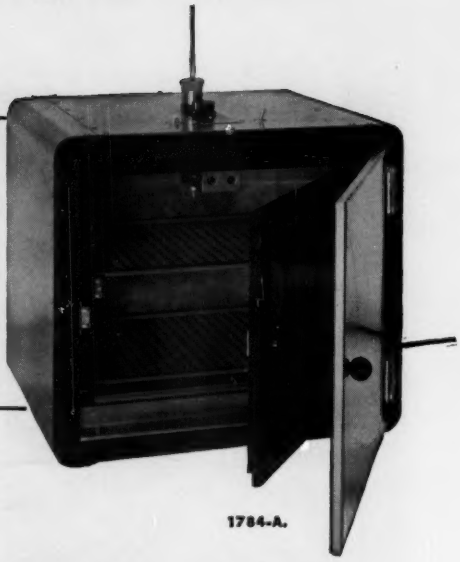

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